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(54) Title: BIODEGRADABLE COMPOSITIONS FOR THE CONTROLLED RELEASE OF ENCAPSULATED SUBSTANCES (57) Abstract Pharmaceutical compositions that enable the release of a physiologically active substance over a prolonged period of time following administration to a patient are described. The pharmaceutical compositions are provided by encapsulation of a physiologically active substance into a matrix comprising biodegradable polymers and lipids. The rate of release of the physiologically active substance from the pharmaceutical composition is controlled by varying the ratio of the polymer to the lipid. The compositions can be stored in an aqueous suspension or as a solid dosage form. The physiologically active substances include small molecules, peptides, proteins, nucleic acids and vaccines. The biodegradable polymers include homopolymers, or random or block copolymers. The lipids include phospholipids, cholesterol and glycerides.		

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Biodegradable Compositions for the Controlled Release of Encapsulated Substances

Background of the Invention

5 The invention relates to lipid/polymer-containing biodegradable pharmaceutical compositions which are designed to provide controlled release of encapsulated physiologically active substances.

 Delivery systems offer the advantage of improved bioavailability and a higher therapeutic index over a prolonged period of time for physiologically active
10 substances. Two major classes of drug-delivery systems are formed from either biodegradable polymers or lipids (Langer, R., *Nature* 392: 5-10, 1998). Polymer-based drug-delivery systems have been developed as microspheres for injection, implants, transdermal patches, and aerosols for inhalation (Domb *et al.*, *Handbook of Biodegradable Polymers*, Harwood Academic Publishers, Amsterdam, 1997;
15 Putney *et al.*, *Nature Biotechnology* 16: 153-157, 1998; Edwards *et al.*, *Science* 276: 1868-1871, 1997). Lipid-based drug delivery systems have been developed as unilamellar, multilamellar (Gregoriadis, *Liposome Technology*, Vols. I, II, III, CRC Press, Boca Raton, Fl., 1993) and multivesicular liposomes (U. S. Patent No. 5,422,120 to Kim; U. S. Patent No. 5,723,147 to Kim *et al.*; U. S. Patent No.
20 5,767,627 to Sankaram *et al.*; U. S. Patent Application No. 08/305,158; U. S. Patent Application Nos. 08/723,583, 08/925,532, 08/792,566, and 08/925,531).

 One of the limitations of using biodegradable polymers is that pharmaceutical compositions such as microspheres prepared from biodegradable polymers require storage under anhydrous conditions due to their susceptibility to
25 hydrolysis. As a result, a typical pharmaceutical package of microspheres for injection consists of one vial with an anhydrous formulation of a biodegradable polymer and a physiologically active substance as a solid dosage form and another vial with an aqueous reconstitution medium (*e.g.*, Lupron Depot, *Physicians Desk Reference*, pp. 2739-2746, Medical Economics Company, Inc., Montvale, NJ,

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1997). The contents of the two vials are mixed immediately prior to injection.

Microspheres are prepared by a single emulsification process (U. S. Patent No. 4,389,330 to Tice *et al.*; U.S. Pat. No. 3,691,090 to Kitajima *et al.*), a double emulsification process (Edwards *et al.*, *Science* 276: 1868-1871, 1997), a phase inversion microencapsulation process (Mathiowitz *et al.*, *Nature* 386: 410-413, 1997), or an atomization-freeze process (Putney and Burke, *Nature Biotechnology* 16: 153-157, 1998). In the single emulsification process, a volatile organic solvent phase containing a biodegradable polymer, an aqueous solution necessarily containing an emulsifier such as polyvinyl alcohol, and a physiologically active substance are homogenized to produce an emulsion. The solvent is evaporated and the resulting hardened microspheres are freeze-dried.

In the double emulsification process, an aqueous solution which may contain a physiologically active substance and an volatile organic solvent phase containing a biodegradable polymer are homogenized to form an emulsion. The emulsion is mixed with another aqueous solution, which contains an emulsifier such as polyvinyl alcohol. Evaporation of the solvent and freeze-drying produces microspheres.

In the phase inversion microencapsulation process, drug is added to a dilute polymer solution in a solvent (*e.g.* dichloromethane) which is then poured rapidly into an unstirred bath of another liquid (*e.g.* petroleum ether) causing nano- and microspheres to form spontaneously. In the atomization-freeze process, micronized solid physiologically active substance is suspended in a solvent phase containing a biodegradable polymer that is then atomized using sonication or air-atomization. This produces droplets that are then frozen in liquid nitrogen. Addition of another solvent in which both the polymer and the drug are insoluble extracts the solvent from the microspheres.

Microspheres prepared by the single-emulsification process or the double-emulsification process methods can be aerosolized (Edwards *et al.*, *Science* 276: 1868-1871, 1997). Addition of dipalmitoyl phosphatidylcholine to the solvent phase increases particle size, porosity, and efficiency of aerosolization and

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decreases the mass density. However, formation of the large porous particles still requires an emulsifier such as polyvinyl alcohol. Sucrose stearate, magnesium stearate, aluminum tristearate, sorbitan fatty esters, and polyoxyethylene fatty ethers have been used as droplet stabilizers in a solvent evaporation method for producing microspheres from acrylic polymers (Yuksel *et al.*, *J. Microencapsulation* 14: 725-733, 1997). Poloxamer 188, or Pluronic F68, has been used as a nonionic surfactant in the primary emulsion, in addition to polyvinyl alcohol in the second aqueous phase, for producing microspheres from poly(lactide) using a double emulsification process (Nihant *et al.*, *Pharm. Res.* 11: 1479-1484, 1994). The hydrophilic additives, 2-hydroxypropyl β -cyclodextrin, methyl β -cyclodextrin, Pluronic F-127, L-tartaric acid dimethyl ester, and a hydrophobic additive, beeswax (which consists of esters of long-chain monohydric alcohols with even-numbered carbon chains, esterified with long-chain acids, also having even numbers of carbon atoms) were used to produce poly(lactide-glycolide) double-layer films (Song *et al.*, *J. Controlled Rel.* 45: 177-192, 1997).

Summary of the Invention

The invention is based on the discovery that biodegradable microspheres can be produced which include both polymer and lipid components, and are free of surfactants introduced into a second aqueous phase of the microspheres. A typical surfactant which heretofore had been introduced into the second aqueous phase is polyvinyl alcohol. Production of the biodegradable microspheres of the invention involves substantial or complete removal of volatile organic solvent from the microsphere walls. The microspheres can be loaded with physiologically active substances, which are released *in vitro* and *in vivo*. The rate of release can be easily controlled by adjustments of the lipid/polymer ratio. Previously available microsphere compositions required a change in the identity of the polymer.

In one aspect, the invention provides a lipid/polymer-containing pharmaceutical composition including a biodegradable microsphere which includes at least one type of biodegradable polymer which is soluble in organic solvents,

and at least one type of lipid. Also included in the composition is a physiologically active substance which is releasable from the biodegradable microsphere. The composition is preferably substantially free of volatile organic solvent, and most preferably substantially free of polyvinyl alcohol. The
5 compositions can be in the form of an aqueous suspension, or alternatively in the form of a solid dosage, such as in the tablet, capsule, wafer, transdermal patch, suture, implant, or suppository form.

The biodegradable polymers can be homopolymers such as polylactides, polyglycolides, poly(p-dioxanones), polycaprolactones, polyhydroxyalkanoates,
10 polypropylenefumarates, polyorthoesters, polyphosphate esters, polyanhydrides, polyphosphazenes, polyalkylcyanoacrylates, polypeptides, or genetically engineered polymers. At the same time, the biodegradable polymers can be copolymers (random or block) such as poly(lactide-glycolides), poly(p-dioxanone-lactides), poly(p-dioxanone-glycolides), poly(p-dioxanone-lactide-glycolides), poly(p-
15 dioxanone-caprolactones), poly(p-dioxanone-alkylene carbonates), poly(p-dioxanone-alkylene oxides), poly(p-dioxanone-carbonate-glycolides), poly(p-dioxanone-carbonates), poly(caprolactone-lactides), poly(caprolactone-glycolides), poly(hydroxyalkanoates), poly(propylenefumarates), poly(orthoesters), poly(ether-
esters), poly(ester-amides), poly(ester-urethanes), polyphosphate esters,
20 polyanhydrides, poly(ester-anhydrides), polyphosphazenes, polypeptides and genetically engineered copolymers. The lipids of the pharmaceutical compositions can be zwitterionic lipids, acidic lipids, cationic lipids, sterols, or triglycerides of many types, including many phospholipids.

The physiologically active substances can be hydrophilic, in which case
25 the compositions desirably also include a triglyceride, or they can be hydrophobic, in which case the composition can be substantially free of triglycerides, or they can be amphipathic. The physiologically active substances can be generally classified as antianginas, antiarrhythmics, antiasthmatic agents, antibiotics, antidiabetics, antifungals, antihistamines, antihypertensives, antiparasitics, antineoplastics,
30 antivirals, cardiac glycosides, cytokines such as erythropoietin, herbicides,

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hormones, immunomodulators, monoclonal antibodies, neurotransmitters, nucleic acids, proteins, radio contrast agents, radionuclides, sedatives, analgesics, steroids, tranquilizers, vaccines, vasopressors, anesthetics, peptides, and combinations thereof.

5 In another aspect, the invention provides a way to vary the rate of release of physiologically active substances by varying the lipid to polymer ratio in the compositions of the invention.

In yet another aspect, the invention provides a method for producing biodegradable lipid/polymer compositions of the invention, including a) forming a
10 water-in-oil type emulsion, which includes a first aqueous phase and a volatile organic phase, b) dispersing the emulsion in a second aqueous phase to form solvent spherules, and c) removing the volatile organic solvent to form the lipid/polymer-containing compositions. A pharmaceutically active substance can be included in the process of making the water-in-oil type emulsion, to produce a
15 pharmaceutical composition. The organic solvent is preferably substantially completely removed.

In a further aspect of the invention, pharmaceutical compositions produced by the above process are provided. If the process involves the use of a hydrophilic pharmaceutically active substance, the process further includes a
20 triglyceride in the process of making the water-in-oil type emulsion.

In a further aspect, the invention provides a method of treating a patient with the pharmaceutical compositions of the invention.

One objective of the present invention is to provide a novel pharmaceutical composition as a drug-delivery system with a physiologically active
25 substance encapsulated within, the composition enabling release of the substance over a prolonged period of time. Another objective is to provide a means of controlling the rate of release of the substance from the composition. Another objective is to provide a means of storing the composition either as a solid dosage form or a semi-solid dosage form.

30 Preparation of microspheres from biodegradable polymers according to

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the prior art requires that polyvinyl alcohol be used in the second aqueous solution of a double emulsification process. When an emulsifier such as polyvinyl alcohol is not included in the second aqueous phase of a double emulsion process, the physiologically active substance cannot be encapsulated in prior art microspheres.

5 The compositions of the present invention do not use polyvinyl alcohol in the second aqueous solution. Polyvinyl alcohol is also not utilized in any other aqueous solution for the pharmaceutical compositions of the present invention. In addition to polyvinyl alcohol, the present compositions do not include sucrose stearate, magnesium stearate, aluminum tristearate, sorbitan fatty esters,
10 polyoxyethylene fatty ethers, Poloxamer 188, Pluronic F68, 2-hydroxypropyl β -cyclodextrin, methyl β -cyclodextrin, Pluronic F-127, or L-tartaric acid dimethyl ester.

Rather, the volatile organic solvent phase contains a mixture of lipids and biodegradable polymer. Higher yield and a greater loading of the
15 physiologically active substance are obtained for the lipid/polymer-containing pharmaceutical compositions of the present invention than for polymer microspheres of the prior art.

A variety of physiologically active substances can be encapsulated into the pharmaceutical compositions of the present invention. The useful substances
20 include small molecules, peptides, proteins and nucleic acids. A variety of biodegradable polymers with different lactide:glycolide ratios and different molecular weights can be used for preparing the pharmaceutical compositions of the present invention.

The lipid composition can be varied in order to optimize yield and
25 loading of the physiologically active substance. Thus, there is no requirement of changing the identity of the polymer to affect the loading, yield or release rate of the physiologically active substances to be released. For hydrophobic physiologically active substances, excellent yields and loading are obtained when the pharmaceutical composition does not contain a triglyceride. For hydrophilic
30 physiologically active substances, yield and loading are excellent when the

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pharmaceutical composition contains a triglyceride.

The pharmaceutical compositions of the present invention afford release of the physiologically active substance into physiological fluids *in vitro* over a sustained period. Encapsulated physiologically active substances are released into
5 human plasma with sustained release characteristics under two different assay conditions. Varying the lipid:polymer ratio in the pharmaceutical composition can control the rate of release of the physiologically active substance. Controlling the release rate allows one to ensure that the concentration of physiologically active substance is constantly within the therapeutic window; that is, within a
10 concentration range that is high enough to be efficacious, but not so high as to be toxic.

The pharmaceutical compositions of the present invention also afford release of the physiologically active substance *in vivo* over a sustained period. Serum concentrations of physiologically active substances determined at various
15 times after administration in the unencapsulated form, encapsulation in a lipid-only composition, encapsulation in a polymer-only composition, and encapsulation in a lipid/polymer-containing composition of the present invention show that the serum concentration of physiologically active substances peaked at a later time, and was higher for the inventive pharmaceutical compositions than that observed for the
20 other three compositions. Alternately, those encapsulated physiologically active substances which inhibit the release of endogenous serum components (for example, hormones, enzymes, proteins, carbohydrates and the like) show a decrease in the serum concentration of the inhibited component which was longer lasting than that observed for unencapsulated physiologically active substance.

25 Microsphere compositions prepared from biodegradable polymers according to the prior art are not suitable for storage in aqueous media since the polymer degrades rapidly upon exposure to hydrous conditions. However, the polymer in the lipid/polymer-containing compositions of the present invention is protected against hydrolysis both in accelerated stability studies with heat and acid
30 stress and under normal storage conditions.

The term "solvent spherule" as used throughout the specification and claims means a microscopic spheroid droplet of organic solvent, within which are multiple smaller droplets of a first aqueous solution. The solvent spherules are suspended and totally immersed in a second aqueous solution. The term

5 Areleasable from biodegradable microspheres@ refers to the condition that upon sufficient biodegradation of the microspheres, the physiologically active substance (encapsulated within the microsphere) is able to exert its physiological effect. Implicit in the definition is the idea that when the substance is not released, its effect is diminished to the extent that a physiological effect is not observable. The

10 physiologically active substances can be released from not only the interior of a microsphere, but also from the microsphere wall (matrix). If the physiologically active substance is attached or appended to the matrix, physical separation of the microsphere and matrix is not required for Arelease from the biodegradable microsphere@. The term Atherapeutically effective@ as it pertains to the

15 compositions of this invention, means that a physiologically active substance present in the microspheres is released in a manner sufficient to achieve a particular level of treatment of a disorder.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art

20 to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present

25 specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Fig. 1 is a plot of the percentage of encapsulated cytarabine from various lipid/polymer-containing pharmaceutical compositions prepared from cytarabine in hydrochloric acid under static conditions versus time *in vitro*.

5 Fig. 2 is a plot of the percentage of encapsulated cytarabine from various lipid/polymer-containing pharmaceutical compositions prepared from cytarabine in water under static conditions versus time *in vitro*.

Fig. 3 is a plot of the percentage of encapsulated cytarabine from various lipid/polymer-containing pharmaceutical compositions prepared from
10 cytarabine in hydrochloric acid under dynamic conditions versus time *in vitro*.

Fig. 4 is a plot of the percentage of encapsulated cytarabine from various lipid/polymer-containing pharmaceutical compositions prepared from cytarabine in water under dynamic conditions versus time *in vitro*.

Fig. 5 is a plot of the serum concentrations of amikacin at different
15 times after oral administration of unencapsulated amikacin, amikacin encapsulated into a lipid-only pharmaceutical composition, a lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio of 1:1, or a polymer-only pharmaceutical composition.

Fig. 6 is a series of size exclusion chromatograms of (A) a solution of
20 polymer-only composition in chloroform, (B) polymer-only composition suspended in sodium chloride, (C) polymer-only composition suspended in hydrochloric acid, and (D) polymer-only composition suspended in hydrochloric acid and heat-stressed.

Fig. 7 is a series of size exclusion chromatograms of a suspension of
25 lipid/polymer-containing pharmaceutical compositions with lipid:polymer ratios of 1:1 by weight (A) stored in sodium chloride, (B) stored in hydrochloric acid, (C) stored in hydrochloric acid and heat-stressed.

Fig. 8 is a series of size exclusion chromatograms of a suspension of

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(A) a freshly prepared lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio of 1:1 by weight, and (B) an identical sample stored in the refrigerator for 9 months.

Fig. 9 is a plot of the change in molecular weight of PLGA in a lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio of 1:1 by weight (Δ) and a polymer-only pharmaceutical composition (O) versus time.

Fig. 10 is a plot of the change in mean particle diameter with the osmolality of the suspending medium for lipid/polymer microspheres of various compositions.

Fig. 11 is a plot of serum testosterone concentrations of varying times after subcutaneous administration of A) a solution of leuprolide in 1.9:1 lipid/polymer preparation, B) free leuprolide and C) 0.9% saline solution.

Fig. 12 is an expanded plot of Fig. 11.

Fig. 13 is a plot of increase in hematocrit at various times after intraperitoneal administration of 500 IU of erythropoietin as A) 1:1 lipid/polymer preparation B) 4:1 lipid/polymer preparation C) free erythropoietin, and D) 1:1 lipid/polymer blank.

Detailed Description

The invention features lipid/polymer-containing compositions that can be loaded with physiologically active substances to provide sustained release *in vitro*, and *in vivo*. The resulting pharmaceutical compositions can be formulated to yield a range of release rates. The pharmaceutical compositions possess excellent yield and loading characteristics for a variety of physiologically active substances.

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Biodegradable Polymer

The lipid/polymer-containing compositions of the invention contain at least one type of biodegradable polymer. The biodegradable polymer may be a homopolymer, or a random or block copolymer, or a blend or physical mixture thereof, with the limitation that the polymer be soluble in volatile organic solvents. Unless the optical activity of a particular material is designated by [L]- or [D]-, the material is presumed to be achiral or a racemic mixture. Meso compounds (those compounds with internally canceling optical activity) are also envisioned as being useful in the present invention.

10 A biodegradable polymer is one that can be degraded to a low molecular weight and may or may not be eliminated from a living organism. The products of biodegradation may be the individual monomer units, groups of monomer units, molecular entities smaller than individual monomer units, or combinations of such products. Such polymers can also be metabolized by organisms. Biodegradable
15 polymers can be made up of biodegradable monomer units. A biodegradable compound is one that can be acted upon biochemically by living cells or organisms, or parts of these systems, or reagents commonly found in such cells, organisms, or systems, including water, and broken down into lower molecular weight products. An organism can play an active or passive role in such processes.

20 The biodegradable polymer chains useful in the invention preferably have molecular weights in the range 500 to 5,000,000. The biodegradable polymers can be homopolymers, or random or block copolymers. The copolymer can be a random copolymer containing a random number of subunits of a first copolymer interspersed by a random number of subunits of a second copolymer.
25 The copolymer can also be block copolymer containing one or more blocks of a first copolymer interspersed by blocks of a second copolymer. The block copolymer can also include a block of a first copolymer connected to a block of a second copolymer, without significant interdispersion of the first and second copolymers.

30 Biodegradable homopolymers useful in the invention can be made up of

monomer units selected from the following groups: hydroxy carboxylic acids such as α -hydroxy carboxylic acids including lactic acid, glycolic acid, lactide (intermolecularly esterified dilactic acid), and glycolide (intermolecularly esterified diglycolic acid); β -hydroxy carboxylic acids including β -methyl- β -propiolactone; γ -hydroxy carboxylic acids δ -hydroxy carboxylic acids; and ϵ -hydroxy carboxylic acids including ϵ -hydroxy caproic acid; lactones such as: β -lactones; γ -lactones; δ -lactones including valerolactone; and ϵ -lactones such as ϵ -caprolactone; benzyl ester-protected lactones such as benzyl malolactone; lactams such as: β -lactams; γ -lactams; δ -lactams; and ϵ -lactams; thiolactones such as 1,4-dithiane-2,5-dione; dioxanones; unfunctionalized cyclic carbonates such as: trimethylene carbonate, alkyl substituted trimethylene carbonates, and spiro-bis-dimethylene carbonate (2,4,7,9-tetraoxa-spiro[5.5]undecan-3,8-dione); anhydrides; substituted N-carboxy anhydrides; propylene fumarates; orthoesters; phosphate esters; phosphazenes; alkylcyanoacrylates; aminoacids; polyhydroxybutyrates; and substituted variations of the above monomers.

The use of such monomers results in homopolymers such as polylactide, polyglycolide, poly(p-dioxanone), polycaprolactone, polyhydroxyalkanoate, polypropylenefumarate, polyorthoesters, polyphosphate esters, polyanhydrides, polyphosphazenes, polyalkylcyanoacrylates, polypeptides, or genetically engineered polymers, and other homopolymers which can be formed from the above mentioned examples of monomers. Combinations of these homopolymers can also be used to prepare the microspheres of the pharmaceutical compositions of the invention.

The biodegradable copolymers can be selected from poly(lactide-glycolide), poly(p-dioxanone-lactide), poly(p-dioxanone-glycolide), poly(p-dioxanone-lactide-glycolide), poly(p-dioxanone-caprolactone), poly(p-dioxanone-alkylene carbonate), poly(p-dioxanone-alkylene oxide), poly(p-dioxanone-carbonate-glycolide), poly(p-dioxanone-carbonate), poly(caprolactone-lactide), poly(caprolactone-glycolide), poly(hydroxyalkanoate), poly(propylenefumarate), poly(ortho esters), poly(ether-ester), poly(ester-amide), poly(ester-urethane), polyphosphate esters, polyanhydrides, poly(ester-anhydride), polyphosphazenes,

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polypeptides or genetically engineered polymers. Combinations of these copolymers can also be used to prepare the microspheres of the pharmaceutical compositions of the invention.

Preferred biodegradable polymers are polylactide, and poly(lactide-glycolide). In some lactide-containing embodiments, the polymer is prepared by polymerization of a composition including lactide in which greater than about 50% by weight of the lactide is optically active and less than 50% is optically inactive, *i.e.*, racemic [D,L]-lactide and meso [D,L]-lactide. In other embodiments, the optical activity of the lactide monomers is defined as [L], and the lactide monomers are at least about 90% optically active [L]-lactide. In still other embodiments, the lactide monomers are at least about 95% optically active [L]-lactide.

Lipids

The lipid/polymer-containing compositions include at least one type of lipid. The lipids may be natural or synthetic in origin and include phospholipids, sphingolipids, sphingophospholipids, sterols and glycerides. The lipids to be used in the compositions of the invention are generally amphipathic, meaning that they have a hydrophilic head group and a hydrophobic tail group, and may have membrane-forming capability. The phospholipids and sphingolipids may be anionic, cationic, nonionic, acidic or zwitterionic (having no net charge at their isoelectric point), wherein the two hydrocarbon chains of the lipids are typically between 12 and 22 carbons atoms in length, and have varying degrees of unsaturation. Preferred anionic phospholipids include phosphatidic acids, phosphatidylserines, phosphatidylglycerols, phosphatidylinositols and cardiolipins. Preferred zwitterionic phospholipids are phosphatidylcholines, phosphatidylethanolamines and sphingomyelins. Preferred cationic lipids are diacyl dimethylammonium propanes, acyl trimethylammonium propanes, and stearylamine. Preferred sterols are cholesterol, ergosterol, lanosterol, or esters thereof. The glycerides can be monoglycerides, diglycerides or triglycerides including triolein, and can have varying degrees of unsaturation, with the limitation that the fatty acid

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hydrocarbon chains of the glycerides are typically between 12 and 22 carbons atoms in length, and have varying degrees of unsaturation. Combinations of these lipids can also be used to prepare the microspheres of the pharmaceutical compositions of the invention.

5 Physiologically active substance

Physiologically active substance means a natural, synthetic or genetically engineered chemical or biological compound that is known in the art as having utility for modulating physiological processes in order to afford diagnosis of, prophylaxis against, or treatment of, an undesired existing condition in a living
10 being. Physiologically active substances include drugs such as antianginas, antiarrhythmics, antiasthmatic agents, antibiotics, antidiabetics, antifungals, antihistamines, antihypertensives, antiparasitics, antineoplastics, antitumor drugs, antivirals, cardiac glycosides, herbicides, hormones, immunomodulators, monoclonal antibodies, neurotransmitters, nucleic acids, proteins, radio contrast
15 agents, radionuclides, sedatives, analgesics, steroids, tranquilizers, vaccines, vasopressors, anesthetics, peptides and the like.

Of particular interest are semisynthetic amino glycoside antibiotics including amikacin; antidiabetics; peptides such as insulin; antitumor drugs including paclitaxel; antineoplastics including cytarabine, 5-fluorouracil, leuprolide,
20 and floxuridine; alkaloid opiate analgesics including morphine and hydromorphone; local anesthetics including bupivacaine, synthetic anti-inflammatory adrenocortical steroids including dexamethasone; antimetabolites including methotrexate; glycopeptide antibiotics including bleomycin; vincalukoblastines and stathmokinetic oncolytic agents including vincristine and vinblastine; hormones
25 such as erythropoietin, plasma proteins, cytokines, growth factors, DNA and RNA from a variety of organisms, and antisense oligonucleotides. Prodrugs which undergo conversion to the indicated physiologically active substances upon local interactions with the intracellular medium, cells, or tissues can also be employed in the invention. Any pharmaceutically acceptable salt of a particular physiologically

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active substance which is capable of forming such a salt is also envisioned as useful in the present invention, including halide salts, phosphate salts, acetate salts, and other salts.

The physiologically active substances may be used alone or in combination with the limitation that the amount of the substance in the pharmaceutical composition be sufficient to enable the diagnosis of, prophylaxis against, or the treatment of, an undesired existing condition in a living being. Generally, the dosage will vary with the age, condition, sex, and extent of the undesired condition in the patient, and can be determined by one skilled in the art.

10 The dosage range appropriate for human use includes a range of 0.1 to 6,000 mg of the physiologically active substance per square meter of body surface area.

The pharmaceutical compositions of the invention can be administered parenterally by injection or by gradual infusion over time. The compositions can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, 15 intracavity, or transdermally. Other methods of administration will be known to those skilled in the art. For some applications, such as subcutaneous administration, the dose required may be quite small, but for other applications, such as intraperitoneal administration, the required dose may be very large. While doses outside the foregoing dosage range may be given, this range encompasses the breadth of use for practically all physiologically active substances. The 20 pharmaceutical compositions of the invention can also be administered enterally.

Double Emulsification Process

The double emulsification process comprises a three-step process producing a semi-solid dosage form of the pharmaceutical composition of the invention. First, a water-in-oil-type emulsion is formed from a first aqueous 25 phase and a volatile organic solvent phase, wherein the emulsion contains a physiologically active substance in either the first aqueous or volatile organic solvent phase and the volatile organic solvent phase includes at least one type of biodegradable polymer or copolymer and at least one type of lipid. If the

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physiologically active substance is hydrophilic, it is present in the first aqueous phase, and if the physiologically active substance is hydrophobic, it is present in the volatile organic solvent phase. Physiologically active substances that are amphipathic can be present in either the first aqueous phase or the volatile organic solvent phase or in both phases.

The Awater-in-oil@-type emulsion is characterized by the presence of disconnected isolated aqueous droplets dispersed in a continuous matrix formed by the volatile organic solvent phase. Ethers, hydrocarbons, halogenated hydrocarbons may be used as the volatile organic solvent. Preferred volatile organic solvents are chloroform, dichloromethane, diethyl ether, isopropyl ether, and combinations thereof. The first aqueous phase may contain excipients such as osmotic spacers, acids, bases, buffers, nutrients, supplements or similar compounds. The Awater-in-oil@-type emulsion can be produced by mechanical agitation such as by ultrasonic energy, nozzle atomization, by the use of static mixers, impeller mixers or vibratory-type mixers. Forcing the phases through a porous pipe to produce uniform sized emulsion particles can also form such emulsions. These methods result in the formation of solvent spherules.

Second, the solvent spherules which are formed from the first Awater-in-oil@-type emulsion are introduced into a second aqueous phase and mixed, analogously as described for the first step. The second aqueous phase can be water, or may contain electrolytes, buffer salts, or other excipients well known in the art of semi-solid dosage forms, and preferably contains glucose and lysine.

In the third step, the volatile organic solvent is removed, generally by evaporation, for instance, under reduced pressure or by passing a stream of gas over or through the spherules. Representative gases satisfactory for use in evaporating the solvent include nitrogen, helium, argon, carbon dioxide, air or combinations thereof. When the solvent is substantially or completely removed, the lipid/polymer-containing composition is formed with the first aqueous solution encapsulated in biodegradable microspheres formed from the polymer and lipid components, with the microspheres suspended in the second aqueous solution.

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If desired, the second aqueous solution may be exchanged for another aqueous solution by washing, centrifugation, filtration, or removed by freeze-drying or lyophilization to form a solid dosage. The solid dosage form of the pharmaceutical composition obtained, by, for example freeze-drying, may be further processed to produce tablets, capsules, wafers, patches, suppositories, sutures, implants or other solid dosage forms known to those skilled in the art.

Pharmaceutical Composition

The pharmaceutical compositions of the invention comprise at least one type of biodegradable polymer or copolymer, at least one type of lipid and at least one physiologically active substance. The biodegradable polymer can take the form of a random copolymer or block copolymer, which can be made up of a variety of monomeric units. For example, if the biodegradable copolymer is made up of lactic acid (or lactide) and glycolic acid (or glycolide), the ratio of lactide to glycolide can range from about 99:1 to about 2:3, preferably from about 10:1 to about 1:1. The biodegradable polymer (in whatever form, homo- or co-polymer) and lipid can be present in a lipid to polymer ratio of from about 20:1 to about 1:5, preferably from about 19:1 to about 1:3.

The biodegradable polymer or copolymer and the lipid form a multicompartmental structure providing multiple non-concentric compartments for encapsulating hydrophilic physiologically active substances; the biodegradable polymer and the lipid forming the boundaries of the compartments. The boundaries can contain hydrophobic or amphipathic physiologically active substances. The pharmaceutical composition can be stored in a solid dosage form or semi-solid dosage form. The term Asemi-solid dosage form@ means an aqueous suspension of the pharmaceutical composition. A semi-solid dosage form can be formed by addition of an aqueous medium to a solid dosage form of the pharmaceutical composition, or can be formed directly by the double emulsion techniques described above. Preferred aqueous media are water, aqueous solutions of sodium chloride, pharmaceutical excipients, and buffered solutions with pH in

the range 2 to 10. Preferred pharmaceutical excipients are phosphate, citrate, acetate, glucuronate, mannitol, polysorbate, carboxymethylcellulose, gelatin and lactate.

The term Asolid dosage form@ includes amorphous powders, tablets, capsules, aerosols, wafers, transdermal patches, suppositories, or implants.

Amorphous powders can be formed by lyophilization of a semi-solid dosage form of the pharmaceutical composition. Tablets, capsules, aerosols, wafers, patches, suppositories, and implants can be formed by techniques well known to those in the art.

The pharmaceutical composition can be administered to a living being by any desired route, for example, intramuscular, intraarticular, epidural, intrathecal, intraperitoneal, subcutaneous, intravenous, intralymphatic, oral, submucosal, transdermal, rectal, vaginal, intranasal, intraocular and by implantation under different kinds of epithelia, including the bronchial epithelia, the gastrointestinal epithelia, the urogenital epithelia, and various mucous membranes of the body.

Characterization of the Pharmaceutical Composition

In order to compare the pharmaceutical compositions in their semi-solid suspension dosage forms, the following parameters are defined. The amount of the physiologically active substance in the suspension is determined by an appropriate assay as described below. The yield of the physiologically active substance is defined by the following equation.

$$\text{Yield (\%)} = \frac{\text{Amount Recovered (mg)}}{\text{Amount Input (mg)}} \cdot 100$$

In the above equation, the amount recovered is the amount of the physiologically active substance determined to be in the biodegradable microspheres of the pharmaceutical composition and the amount input is the total amount of the physiologically active substance used in the preparation of the pharmaceutical composition.

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The suspension is centrifuged in hematocrit capillary tubes to produce a pellet fraction and a supernatant fraction. Using the standard technique of hematocrit measurement, the relative volumes of the pellet and of the suspension are given by the distance from the bottom of the pellet to the top of the pellet, and from the bottom of the pellet to the top of the supernatant, respectively. The ratio of the relative volume of the pellet and the relative volume of the suspension is defined as the pellet fraction and is given by the following equation.

$$\text{Pellet Fraction} = \frac{\text{Volume of the Pellet}}{\text{Volume of the Suspension}}$$

The unencapsulated fraction is defined as the fraction of the physiologically active substance in the pharmaceutical composition that is not encapsulated and resides outside of the biodegradable microspheres in the suspending medium, and is given by the following equation.

$$\text{Unencapsulated Fraction} = \frac{\text{Amount in the Supernatant (mg)}}{\text{Amount in the Suspension (mg)}}$$

The encapsulated fraction is one minus the unencapsulated fraction. Loading is defined as the concentration of the physiologically active substance in the pellet fraction of the suspension, and is given by the following equation.

$$\text{Loading (mg/mL)} = \frac{\text{Encapsulated Fraction}}{\text{Pellet Fraction}} \cdot \text{Concentration in Suspension (mg/mL)}$$

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

20

EXAMPLES

The following examples illustrate the preparation and properties of the biodegradable lipid/polymer-containing pharmaceutical compositions of the present invention.

Example 1: Preparation of the Pharmaceutical Composition

25

The pharmaceutical compositions shown in Table 1 were prepared by

the double emulsification process. The aqueous phase contained amikacin (Bristol-Myers Squibb, Syracuse, NY) at the concentrations shown in Table 1 and a pH of 8. Additionally, for comparative formulations, the aqueous phase contained 4% polyvinyl alcohol (Sigma Chemical Co.) as indicated in Table 1.

5 The solvent phase contained either the polymer poly(DL-lactide-glycolide) (PLGA) alone at a concentration of 250 mg/mL, or a mixture of PLGA, dioleoyl phosphatidylcholine (DOPC), dipalmitoyl phosphatidylglycerol (DPPG), cholesterol and triolein at concentrations of 22.4 mg/mL, 10.4 mg/mL, 2.1 mg/mL, 7.7 mg/mL, and 2.2 mg/mL, respectively. PLGA was from Sigma Chemical
10 Company (St. Louis, MO), with a lactide:glycolide ratio of 50:50 and a molecular weight of 40,000-75,000. This material (with lactide:glycolide of 1:1) was used in all experiments, except where indicated (in Example 4). DOPC, DPPG, and triolein were from Avanti Polar Lipids (Alabaster, AL), and cholesterol was from Spectrum Chemical Manufacturing Corp. (Gardena, CA). This corresponds to a
15 solvent phase either with polymer alone, or with lipids and polymer in a 1:1 weight ratio. The ratios of the volume of the aqueous phase to the volume of the volatile organic solvent phase are given in Table 1. The Awater-in-oil® emulsion was prepared by mixing on a Homo Mixer (Tokushu Kika Kogyo Co., Ltd., Osaka, Japan) at a speed of 9,600 rpm for 20 min. The second aqueous phase was either
20 4% wt. polyvinyl alcohol (PVA) for comparative formulations, water, or a mixture of 5% wt. glucose and 40 mM lysine. Spherules were formed by mixing at 4,000 rpm for 1 min. The suspending medium was exchanged with 0.9% wt. sodium chloride by washing and centrifuging at 600xg twice.

The concentration of amikacin in the pharmaceutical composition, and
25 the supernatant of the pharmaceutical composition were determined by capillary electrophoresis (3D CE model 1600, Hewlett-Packard, Irvine, CA) using a fused silica column (50 Fm inner diameter, 50 cm length, Hewlett-Packard, Irvine, CA), and 0.1 M phosphate, pH 2.5 (Peptide Separation Buffer, Sigma Chemical Co.) as the running buffer. The yield of the physiologically active substance and drug
30 loading are summarized in Table 1.

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The mean particle diameter was determined on a laser scattering particle size distribution analyzer (Model LA-910, Horiba Instruments, Irvine, CA) using the length-weighted distribution base and a relative refractive index of 1.18-1.00i. The mean particle diameters were 43.6 Fm for the polymer pharmaceutical
5 composition prepared with 4 wt% polyvinyl alcohol (comparative formulation) in the second aqueous phase, and 9.0 Fm for the lipid/polymer-containing pharmaceutical composition of the present invention in Table 1 with a lipid:polymer ratio of 1:1.

Table 1: Characteristics of Polymer-only and Lipid/Polymer-Containing Pharmaceutical Compositions.

	Aqueous Phase		Solvent Phase		Aqueous: Solvent Phase Volume Ratio (mL:mL)	Second Aqueous Phase	Yield (%)	Loading (mg/mL)
	Amikacin, mg/mL	Excipients	Polymer	Lipids				
5	320	None	PLGA	None	5:0.5	4 wt% PVA	31	20.8
	320	None	PLGA	None	5:0.5	H ₂ O	0	0
	320	None	PLGA	None	5:0.5	5 % Glucose, 40 mM Lysine	0	0
	80	4% PVA	PLGA	None	5:5	H ₂ O	0	0
10	80	4% PVA	PLGA	None	5:5	5% Glucose, 40 mM Lysine	0	0
	80	None	PLGA	DOPC, DPPG, Cholesterol, Triolein	5:5	5% Glucose, 40 mM Lysine	64	65.9

The results in Table 1 show that addition of an emulsifier such as polyvinyl alcohol is required for the preparation of polymer-only microspheres containing physiologically active substance. They also show that addition of the emulsifier to the second aqueous phase is an absolute requirement for the encapsulation of physiologically active substance for polymer-only microspheres. The pharmaceutical composition of the present invention (last entry of Table 1) does not use such an emulsifier, in any of the aqueous phases. Instead, encapsulation is achieved by addition of lipids to the volatile organic solvent phase.

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Therefore, the lipid/polymer-containing pharmaceutical compositions of the present invention are quite different from the compositions of the prior art.

Additionally, Table 1 demonstrates the high loading which is achieved with the pharmaceutical compositions of the invention, even considering a four-fold
5 lower concentration of pharmaceutically active composition in the first aqueous phase.

Example 2: Preparation of Pharmaceutical Compositions Containing
Physiologically Active Substances.

The pharmaceutical compositions were prepared by employing a double
10 emulsification process. The Awater-in-oil@ type emulsion was prepared from 5 mL of an aqueous phase containing the physiologically active substance and excipients the concentrations of which are specified in Table 2, and 5 mL of an volatile organic solvent phase, containing lipids and polymer at a ratio of 1:1 and was prepared as described in Example 1. The physiologically active substances
15 were cytarabine (Pfanstiehl, Waukegan, IL), bovine zinc insulin (Sigma Chemical Co., St. Louis, MO), herring sperm DNA (Gibco BRL, Gaithersburg, MD), or dexamethasone phosphate (Sigma Chemical Co., St. Louis, MO). The mixing speed and time for producing the Awater-in-oil@ type emulsion were 9,600 rpm for 20 min. The second aqueous phase was 30 mL of a solution containing 5%
20 glucose and 40 mM lysine. Spherules were formed by mixing at 4,000 rpm for 1 min. The suspending medium was exchanged with 0.9% wt. sodium chloride by washing and centrifuging at 600xg twice.

The concentration of cytarabine in the pharmaceutical composition, and the supernatant of the pharmaceutical composition were determined by isocratic
25 reverse phase HPLC (Hewlett-Packard, Wilmington, DE) using a C18 column (Vydac, Hesperia, CA), 10 mM potassium phosphate, pH 2.8 (J.T. Baker, Phillipsburg, NJ) as the mobile phase, a flow rate of 1 mL/min and 280 nm for the wavelength of detection.

The concentration of insulin in the pharmaceutical composition, and the supernatant of the pharmaceutical composition were determined by isocratic reverse phase HPLC using a C18 column, a solution of 50% by volume of acetonitrile (Burdick & Jackson), 50% water and 0.1% trifluoroacetic acid (Pierce, Rockford, IL) as the mobile phase, a flow rate of 1 mL/min and 280 nm for the wavelength of detection.

The concentration of herring sperm DNA in the pharmaceutical composition, and the supernatant of the pharmaceutical composition were determined by measuring absorbance at 260 nm on a spectrophotometer (Model U-2000, Hitachi, Danbury, CT) of the pharmaceutical composition dissolved in acidic isopropanol relative to that of a standard solution of herring sperm DNA of known concentration.

The concentration of dexamethasone in the pharmaceutical composition, and the supernatant of the pharmaceutical composition were determined by isocratic reverse phase HPLC using a C18 column (5Fm, 4.6 x 250 mm, Vydac), a mixture of 60% by volume methanol and 40% of 50 mM potassium phosphate, pH 2.8 as the mobile phase, a flow rate of 0.5 mL/min and 254 nm for the wavelength of detection.

The yield of the physiologically active substance, drug loading and the mean particle diameter are summarized in Table 2.

Table 2: Characteristics of Pharmaceutical Compositions containing Physiologically Active Compounds

Physiologically Active Substance	Aqueous Phase		Yield (%)	Mean Particle Diameter (Fm)	Drug Loading (mg/mL)
	Concentration (mg/mL)	Excipients			
Cytarabine	30	136 mM HCl	70	17	25.0
Cytarabine	75	None	62	14	59.6
Insulin	20	5% Dextrose, 50 mM HCl	38	13	10.7

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Herring Sperm DNA	1	None	45	14	0.16
Dexamethasone phosphate	15	None	14	7	13.4

5 Example 3: Preparation of Pharmaceutical Compositions with Different Lipid:polymer Ratios

The pharmaceutical compositions were prepared as described in Example 2, with the following modifications. The aqueous phase contained either 30 mg/mL cytarabine and 136 mM hydrochloric acid, or 20 mg/mL bovine zinc
10 insulin, 5% dextrose and 50 mM hydrochloric acid. A series of volatile organic solvent phases with lipid:polymer ratios of 1:0, 19:1, 4:1, 3:2, 1:1 or 1:3 were prepared by mixing in varying proportions a chloroform solution containing dioleoyl phosphatidylcholine, cholesterol, dipalmitoyl phosphatidylglycerol, and triolein at concentrations of 20.8 mg/mL, 15.4 mg/mL, 4.2 mg/mL and 4.4 mg/mL,
15 respectively, with a chloroform solution of the polymer at a concentration of 44.8 mg/mL. In Table 3, the first aqueous solution was a mixture of 30 mg/mL cytarabine and 136 mM hydrochloric acid. In Table 4, the first aqueous solution was a mixture of 20 mg/mL insulin, 50 mM hydrochloric acid and 5 wt% dextrose. The concentrations of cytarabine and insulin were determined by assays
20 as described in Example 2.

The yield, mean particle diameter and drug loading for the pharmaceutical compositions containing cytarabine are given in Table 3, and the characteristics for the pharmaceutical compositions containing insulin are in Table 4.

25 Table 3: Characteristics of Cytarabine-containing Pharmaceutical Compositions Containing Differing Lipid-Polymer Ratios.

Lipid:Polymer	Yield (%)	Mean Particle Diameter, Fm	Drug Loading (mg/mL)
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	1:0	56	15	43.5
	19:1	58	13	34.6
	4:1	78	11	35.5
	3:2	80	14	34.1
5	1:3	31	15	21.7

Table 4: Characteristics of Insulin-containing Pharmaceutical Compositions Containing Differing Lipid-Polymer Ratios.

	Lipid:Polymer	Yield (%)	Mean Particle Diameter, Fm	Drug Loading (mg/mL)
	1:0	68	17	29.4
10	4:1	100	16	22.5
	3:2	83	14	21.8
	1:1	38	13	10.7

As the results in Tables 3 and 4 demonstrate, high yields of physiologically active substances are generally achieved through use of the lipid/polymer-containing compositions of the invention. The mean particle diameters are typically between 5 and 25 μm , and are usually between 10 and 20 μm .

Example 4: Preparation of Pharmaceutical Compositions with Differing Lipid:Polymer Ratios and with Polymers of Differing Molecular Weight and Differing Lactide:Glycolide

The pharmaceutical compositions were prepared by employing a double emulsification process. The Awater-in-oil@ type emulsion was prepared from an aqueous phase containing the physiologically active substance cytarabine at a concentration of 30 mg/mL in 136 mM hydrochloric acid, and an volatile organic solvent phase containing dioleoyl phosphatidylcholine, cholesterol, dipalmitoyl

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phosphatidylglycerol, triolein, and a poly (lactide-glycolide) polymer (Alkermes, Inc., Blue Ash, OH), as presented in Example 3. The yields of the physiologically active substance, mean particle diameter and drug loading for the pharmaceutical composition with polymers of different lactide:glycolide ratios and varying lipid:polymer ratios are summarized in Tables 5, 6 and 7, respectively.

Table 5: Yield of Physiologically Active Substance Obtained with Differing Molecular Weight and Lactide:Glycolide.

	Lactide : Glycolide Ratio	Polymer Molecular Weight	Yield (%) at Different Lipid:Polymer Ratios			
			4:1	3:2	1:1	0:1
10	100:0	128,450	50	54	56	0
	85:15	149,000	55	56	51	0
	75:25	130,132	43	58	60	0
	65:35	141,000	49	50	55	2
	50:50	69,587	51	63	54	2

Table 6: Mean Particle Diameter of Pharmaceutical Compositions Obtained with Differing Molecular Weight and Lactide:Glycolide.

	Lactide : Glycolide Ratio	Polymer Molecular Weight	Mean Particle Diameter (Fm) at Different Lipid:Polymer Ratios			
			4:1	3:2	1:1	0:1
	100:0	128,450	12	11	11	ND
20	85:15	149,000	12	11	11	4
	75:25	130,132	15	11	11	ND
	65:35	141,000	12	10	10	77
	50:50	69,587	11	10	11	5

ND - Not Determined

Table 7: Loading of Physiologically Active Substance Into Pharmaceutical

Compositions Obtained with Differing Molecular Weight and Lactide:Glycolide.

Lactide : Glycolide Ratio	Polymer Molecular Weight	Loading in the Pharmaceutical Composition (mg/mL) at Different Lipid:Polymer Ratios			
		4:1	3:2	1:1	0:1
100:0	128,450	44.9	43.0	38.6	0
85:15	149,000	46.9	47.0	40.0	0
75:25	130,132	45.2	46.7	32.8	0
65:35	141,000	46.2	41.0	34.7	4.5
50:50	69,587	42.0	45.5	38.7	7.7

The results in Tables 5, 6 and 7 demonstrate that the inventive pharmaceutical compositions display generally high yields and uniform loading of physiologically active substance regardless of the lactide:glycolide ratio, molecular weight of biodegradable polymer, or lipid:polymer ratio, as long as some lipid is present in the compositions. The pharmaceutical compositions of the invention have remarkably similar mean particle diameters, all generally falling between 5 and 25 μm , and usually between 10 and 20 μm .

Example 5: Pharmaceutical Composition Containing a Hydrophobic Physiologically Active Substance with and without a Triglyceride

Two pharmaceutical compositions, one with triglyceride and another without triglyceride, were prepared as generally described in Example 2 with the following modifications. The hydrophobic physiologically active substance was paclitaxel. For the composition with triglyceride, the chloroform solution contained poly(lactide-glycolide), DOPC, DPPG, cholesterol, and triolein (the triglyceride) at concentrations given in Example 2, and paclitaxel (ICN Biochemicals, Aurora, OH) at a concentration of 5 mg/mL. For the composition without triglyceride, the chloroform solution contained poly(lactide-glycolide), DOPC, DPPG and cholesterol at the concentrations given in Example 2 and paclitaxel at a

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concentration of 5 mg/mL. For both compositions, the lipid:polymer ratio was 1:1. For both compositions, 5 mL of an aqueous solution containing 10% wt. sucrose (Pfanstiehl, Waukegan, IL) was used to prepare the Awater-in-oil@ type emulsion.

The concentration of paclitaxel in the pharmaceutical compositions was
5 determined by reverse phase HPLC with a C18 column (5 Fm, 4.6 x 250 mm, 90 A, Vydac), a water (Mallinckrodt, Paris, KY):acetonitrile gradient with a flow rate of 1 mL/min and using 273 nm as the wavelength of detection. Yield, particle size and drug loading for both compositions are shown in Table 8.

10 **Table 8: Characteristics of Pharmaceutical Compositions Containing Paclitaxel in the Presence and Absence of Triglyceride.**

Composition	Yield (%)	Mean Particle Diameter (Fm)	Drug Loading (mg/mL)
With Triglyceride	52	16	4.0
Without Triglyceride	53	16	12.1

This example shows that significantly higher loading of hydrophobic
15 physiologically active substances is obtained when a triglyceride is omitted from the composition.

Example 6: Pharmaceutical Composition Containing a Hydrophilic Physiologically Active Substance with and without a Triglyceride

Two pharmaceutical compositions, one with triglyceride and another without triglyceride, were prepared as generally described in Example 2 with the following modifications. The hydrophilic physiologically active substance was amikacin. For the composition with triglyceride, the chloroform solution contained poly(lactide-glycolide), DOPC, DPPG, cholesterol, and triolein (the triglyceride) at concentrations given in Example 2. For the composition without triglyceride, the chloroform solution contained poly(lactide-glycolide), DOPC, DPPG and cholesterol at the concentrations given in Example 2. For both compositions, the lipid:polymer ratio was 1:1. For both compositions, 5 mL of an aqueous solution containing 80 mg/mL amikacin, pH 8 was used to prepare the water-in-oil emulsion. Yield, particle size and drug loading for both compositions are shown in Table 9.

Table 9: Characteristics of pharmaceutical compositions containing amikacin in the presence and absence of a triglyceride.

Composition	Yield (%)	Mean Particle Diameter (Fm)	Drug Loading (mg/mL)
With Triglyceride	64	9	65.9
Without Triglyceride	0	4	0

This example shows that loading of hydrophilic physiologically active substances does not occur when a triglyceride is excluded from the composition. In contrast significant loading of hydrophilic physiologically active substances occurs with triglyceride present in the composition.

Example 7: Sustained Release *in vitro* of Encapsulated Physiologically Active Substance from Pharmaceutical Composition

In vitro release studies were performed in human plasma under static and dynamic conditions. Two sets of pharmaceutical compositions for *in vitro*

release studies were prepared as described in Example 2 with the following modifications. One set was prepared from an aqueous phase containing 30 mg/mL cytarabine in 136 mM hydrochloric acid. Another set was prepared from an aqueous phase containing 75 mg/mL cytarabine in water. For each set, three compositions were prepared as described in Example 3 with lipid:polymer ratios of 4:1, 3:2 and 1:1. For all six pharmaceutical compositions, the concentration of cytarabine was adjusted to 10 mg/mL. The pharmaceutical composition (1 mL) was mixed with type O human plasma (24 mL) (NABI Biomedical, Scottsdale, AZ) and 1% wt. sodium azide (150 FL) (Sigma Chemical Co., St Louis, MO).

For the *in vitro* release studies under static conditions, aliquots of 1.5 mL in triplicate were placed in vials which were incubated at 37 EC for different time periods extending up to 14 days. For the *in vitro* release studies under dynamic conditions, the vials were placed on a rotator (Hematology/Chemistry Mixer, Model 346, Fisher Scientific, Pittsburgh, PA) continuously rotating at 16 rpm. The platform was placed in an incubator at 37 EC for different time periods extending up to 14 days.

At each time point, 10 mL of 0.9% sodium chloride were added to an aliquot and the resulting suspension was centrifuged for 10 min. at 600xg. The supernatants were discarded and the pellets were solubilized in a solution of 12:3:1 acetonitrile:isopropyl alcohol:1N hydrochloric acid. The amount of the physiologically active substance in the solubilized pellet was determined as described in the assay for cytarabine (see Example 2). The percentage encapsulated drug in Figs. 1, 2, 3 and 4 is defined by the following equation.

$$\text{Encapsulated Drug (\%)} = \frac{\text{Amount in the Pellet (mg)}}{\text{Amount in the Pellet at Time Zero (mg)}} \cdot 100$$

The *in vitro* release profiles in plasma for the lipid/polymer-containing pharmaceutical compositions of lipid:polymer ratios 4:1, 3:2 and 1:1 with 30 mg/mL cytarabine in 136 mM hydrochloric acid under static conditions for the *in vitro* release assay are shown in Fig. 1. The corresponding release profiles for the 4:1, 3:2 and 1:1 lipid/polymer-containing pharmaceutical compositions containing

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75 mg/mL cytarabine in water obtained under static conditions are shown in Fig. 2. The release profiles for the lipid/polymer-containing pharmaceutical compositions measured under dynamic conditions are shown in Figs. 3 (30 mg/mL cytarabine in 136 mM hydrochloric acid) and 4 (75 mg/mL cytarabine in water).

5 These data show that sustained release of the encapsulated physiologically active substance into physiological media is obtained for both compositions under different assay conditions. They also show that the sustained release can be controlled by varying the lipid:polymer ratio.

10 Example 8: Sustained Release *in vivo* of Encapsulated Physiologically Active Substance from Pharmaceutical Composition after a Single Oral Administration

 A lipid-only pharmaceutical composition and a lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio 1:1 were prepared as in Example 1 with the following modifications. The aqueous solution contained 80 mg/mL amikacin, pH 8. For preparing the lipid/polymer-containing
15 pharmaceutical composition with lipid to polymer ratio of 1:1 by weight, the chloroform solution was as described in Example 2. For the lipid-only pharmaceutical composition, the chloroform solution contained DOPC, DPPG, cholesterol and triolein at the concentrations specified in Example 2, without the polymer. The mean particle diameters for the polymer-only and the 1:1
20 lipid/polymer-containing pharmaceutical compositions with a lipid to polymer ratio of 1:1 by weight were 8.4 and 7.8 μ m, respectively.

 For preparation of the polymer-only pharmaceutical composition, 5 mL of a chloroform solution containing poly(lactide-glycolide) at a concentration of 250 mg/mL, and 0.5 mL of an aqueous solution containing 320 mg/mL amikacin,
25 pH 8 were used. The second emulsion was formed by the addition of 20 mL of a 4% polyvinyl alcohol, and mixing at 4000 rpm for 1 minute. The contents of the mixing vessel were poured into a 1 liter Erlenmeyer flask containing 30 mL 4% polyvinyl alcohol. A stream of nitrogen gas set at 50 psi for 20 minutes at 37°C was flushed through the flask to evaporate the chloroform. The mean particle

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diameter for the polymer pharmaceutical composition was 42.1 Fm.

The lipid-only, 1:1 lipid/polymer and the polymer-only pharmaceutical compositions with amikacin encapsulated therein, or unencapsulated amikacin as a solution at pH 8 was administered orally by gavage to 300g male Harlan Sprague
5 Dawley albino rats. The number of animals per group was six. The dose was 20 mg per rat and the volume was 2 mL. Serum was collected via venipuncture (saphenous vein) from unanesthetized animals at 15, 45, 90 min., 3, 8, 24, and 48 hours. The specimens were kept at -40°C until analysis.

The concentration of amikacin in serum was determined using a
10 competitive particle concentration fluorescence immunoassay. Polystyrene beads (Idexx Research Product Division, Memphis, TN) were first coated with anti-amikacin antibody (The Binding Site, San Diego, CA). The beads were incubated with a combination of fluorescent-labeled amikacin (The Binding Site) and serum containing amikacin which compete for the anti-amikacin antibody sites on the
15 beads. The amount of fluorescent-labeled amikacin bound to the beads was measured by a fluorescence concentration analyzer (Idexx Research Product Division) which is inversely proportional to the amikacin concentration in serum.

The concentration of amikacin (Fg/mL) in serum at different time points after oral administration for the four treatment groups is shown in Fig. 5. As seen
20 in this figure, the peak circulatory levels were reached at 45 minutes for unencapsulated amikacin, the lipid, and the polymer pharmaceutical compositions, while for the 1:1 lipid/polymer-containing pharmaceutical composition, the peak level was prolonged to 90 minutes. This result demonstrates that use of the lipid/polymer-containing pharmaceutical composition results in a longer duration of
25 release for the encapsulated physiologically active substance. Further, the concentration of amikacin in circulation reached peak levels of 1.82 ± 0.73 , 1.25 ± 0.32 and 0.87 ± 0.13 Fg/mL for unencapsulated amikacin, the lipid, and the polymer pharmaceutical compositions respectively, while for the 1:1 lipid/polymer-containing pharmaceutical composition, a much greater peak level of 2.77 ± 0.39
30 Fg/mL was obtained. This result shows that the bioavailability of the

physiologically active substance is enhanced by use of the lipid/polymer-containing pharmaceutical composition.

Example 9: Stability of Polymers upon Storage in Aqueous Medium

The effect of exposure to an aqueous environment, accelerated by heat
5 and acid on the molecular weight of the polymer, poly(lactide-glycolide), alone or
when it is present in the lipid/polymer-containing composition, was determined by
size exclusion chromatography. A lipid/polymer-containing pharmaceutical
composition with a lipid:polymer ratio of 1:1 by weight and cytarabine as the
physiologically active substance was prepared as described in Example 3. The
10 pellet fraction was adjusted to be 0.2. The suspending medium was 0.9% sodium
chloride.

The average molecular weight of the polymer was determined by size
exclusion chromatography using an evaporative light scattering detector (Richard
Scientific, Laurel, MD), two serially coupled gel filtration columns (Phenogel 10³
15 and 10⁴ A, 7.8 x 300 mm, Phenomenex, Torrance, CA), and chloroform as the
mobile phase at a flow rate of 1 mL/min. The calibration curve was generated
using 1 mg/mL chloroform solutions of polystyrene standards of molecular weight
12600, 20800, 37400, 53500, 79000, and 95050 (American Polymer Standards
Corporation, Mentor, OH). The average molecular weight was calculated
20 following standard procedures: Standard test method for molecular weight averages
and molecular weight distribution by liquid exclusion chromatography (GEL
permeation chromatography - GPC) (American Society for Testing and materials. D
3536-91. Pp. 352-362. 1992; Standard test method for molecular weight averages
and molecular weight distribution of polystyrene by high performance size-
25 exclusion chromatography. American Society for Testing and Materials. D 5296-92.
Pp. 1-14. 1992).

For the polymer-only control composition, a 2 mg/mL solution of
poly(lactide-glycolide) in chloroform was prepared. Samples for determining the
effect of exposure to an aqueous medium, and for acid and heat stress studies were

- 35 -

prepared as follows. A 1 mg/mL solution of the polymer was dried in a vacuum desiccator in order to form a dry residue in a glass vial. Normal saline (750 FL of 0.9% sodium chloride) was added to the glass vial. This sample was used to determine the effect of exposure to an aqueous medium on polymer stability. To
5 determine the effect of acid, 1 mg of the dry residue of the polymer formed in a glass vial was dispersed in 750 FL of 1 N hydrochloric acid and incubated for 1 hour. To determine the effect of acid and heat, 1 mg of the dry residue formed in a glass vial was dispersed in 750 FL of 1 N hydrochloric acid and placed in a boiling water bath for 1 hour. After the samples were incubated as described
10 above, the polymer was extracted by adding 750 FL of chloroform. The chloroform layer was dried in a vacuum desiccator. The dry residue was taken up into chloroform to give a concentration of 2 mg/mL for size exclusion chromatography. Size exclusion chromatograms of a solution of the polymer in chloroform (curve A), the polymer suspended in saline (curve B), the suspension of
15 the polymer suspended in 1 N hydrochloric acid (curve C), and the suspension of the polymer in 1 N hydrochloric acid further stressed by heat (curve D) are shown in Fig. 6. As seen in Fig. 6, the retention time of the polymer is increased upon addition of saline, or when the polymer is subjected to heat or acid, indicating a degradation of the polymer into smaller molecular weight fragments.

20 In order to determine the degradation of the polymer when it is present in the lipid/polymer-containing compositions of the present invention under acid and heat stressed conditions, 10 mL of a lipid/polymer-containing pharmaceutical composition with lipid to polymer ratio of 1:1 by weight containing cytarabine prepared as described in Example 2 with a pellet fraction of 0.2, suspended in 0.9%
25 sodium chloride, were centrifuged at 600 x g. To determine the effect of acid, most of the supernatant (7.5 mL) was discarded and replaced with 7.5 mL of 1 N hydrochloric acid and incubated at ambient temperature for 1 hr. To determine the effect of acid and heat, the suspension in 1 N hydrochloric acid was heated in a boiling water bath for 1 hr. Size exclusion chromatograms of the unstressed
30 sample stored as a suspension in 0.9% sodium chloride (curve A), the sample

stressed by acid (curve B), and the sample stressed by acid and heat (curve C) are shown in Fig. 7. As seen in Fig. 7, the retention time of the polymer is largely unaffected upon adding acid, or heat, indicating that the polymer is protected against hydrolysis by the presence of the lipid in the pharmaceutical compositions.

5 In order to determine polymer degradation under normal storage conditions, a lipid/polymer-containing pharmaceutical composition with a 1:1 lipid:polymer ratio by weight and cytarabine as the physiologically active substance was prepared as described in Example 2. The pharmaceutical composition was stored as a suspension in 0.9% sodium chloride in a refrigerator (2-8 EC) for 9
10 months. The size exclusion chromatograms of a freshly prepared lipid/polymer-containing pharmaceutical composition (curve A) and of a 9 month old composition (curve B) are shown in Fig. 8. As seen in Fig. 8, the polymer has not undergone significant hydrolysis during normal storage conditions.

Another accelerated stability study was conducted in order to compare
15 the change in molecular weight of the polymer as a function of time of incubation at 37 EC for a lipid/polymer-containing pharmaceutical composition with lipid to polymer ratio of 1:1 by weight and a polymer-only formulation with no lipid. The lipid/polymer-containing pharmaceutical composition contained a physiologically active substance, cytarabine, and was prepared as described in Example 3. The
20 polymer-only formulation also contained cytarabine and was prepared as described in Example 8, except that the first aqueous medium contained 150 mg/mL cytarabine in water. The average molecular weight of each polymer at different times of incubation at 37 EC was calculated as described above.

The results of this study, shown in Fig. 9 as circles for the polymer-only
25 pharmaceutical composition and as triangles for the 1:1 lipid/polymer-containing pharmaceutical composition of the present invention, indicate that the rate of polymer degradation is slower for the lipid/polymer-containing pharmaceutical composition than for the polymer-only pharmaceutical composition.

Example 10: Osmotic Sensitivity of Pharmaceutical Composition

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A polymer-only pharmaceutical composition and a lipid/polymer-containing pharmaceutical composition with lipid-polymer ratio, 9:1, 1:1, or 1:3 were prepared as in Example 1 and Example 8. For preparing the lipid/polymer-containing pharmaceutical composition with lipid to polymer ratio of 9:1, 1:1, and 1:3 by weight, the chloroform solution was as described in Example 2.

Sodium Chloride (Fisher Scientific) was added to water in varying amounts which resulted in solutions with the following osmolalities: 57, 116, 180, 269, 341, 422 mOsm/kg. The lipid/polymer containing pharmaceutical composition with lipid-polymer ratio 9:1, 1:1, and 1:3 or the polymer-only pharmaceutical composition were added into each one of the sodium chloride solutions. The particle diameters were measured using a laser scattering particle size distribution analyzer (Model LA-910, Horiba Instruments, Irvine, CA) using the length weighted distribution base and a relative refractive index of 1.18-1.00i. The mean particle diameters as a function of osmolality are shown in Fig. 10.

Fig. 10 shows that the size of the lipid/polymer microsphere compositions shows a dependence on the osmolality of the suspending medium. The size of polymer-only microsphere is independent of osmotic stress under these conditions, however.

Example 11: Sustained Release *in vivo* of Encapsulated Physiologically Active Substance from Pharmaceutical Composition after Subcutaneous Administration

A lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio 1.9:1 was prepared as in Example 1 with the following modifications. The aqueous solution contained 15 mg/mL leuprolide. For preparing the lipid/polymer-containing pharmaceutical composition with lipid to polymer ratio of 1.9:1 by weight. The chloroform solution is as described in Example 2. The chloroform solution contained the polymer poly(DL-lactide) (PLA) at a concentration of 37.22 mg/mL, DEPC, DPPG, cholesterol and triolein at concentrations of 35.6 mg/mL, 6.225 mg/mL, 23.1 mg/mL, 6.5 mg/mL, respectively. PLA was from Sigma Chemical Company (St. Louis, MO), with a

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molecular weight of 106,000. A separate solution of leuprolide contained 6.0 mg/mL in water. DEPC, DPPG, and triolein were from Avanti Polar Lipids (Alabaster, AL) and cholesterol was from Spectrum Chemical Manufacturing Corp. (Gardena, CA). The leuprolide was from Bachem (Torrance, CA). The ratio of the volume of aqueous phase to the volume of volatile organic solvent phase was 5 mL to 5mL. The Awater-in-oil@ emulsion was prepared by mixing on a Homo Mixer (Tokushu Kika Kogy Co., Ltd., Osaka, Japan) at a speed of 9000 rpm for 8 min. The second aqueous phase was a mixture of 5wt.% glucose and 40 mM lysine. Spherules were formed by mixing at 4000 rpm for 1 min. A stream of nitrogen gas set at 50 psi for 20 minutes at 37 EC was flushed through the suspension of spherules to evaporate the chloroform. The suspending medium was exchanged with 0.9wt % sodium chloride by washing and centrifuging at 600xg twice.

The mean particle diameter was determined on a laser scattering particle size distribution analyzer (Model LA-910, Horiba Instruments, Irvine, CA). The mean particle diameter for the lipid/polymer pharmaceutical composition was 15.9 Fm.

The concentration of leuprolide in the pharmaceutical composition, and the supernatant of the pharmaceutical composition were determined by high pressure liquid chromatography (HPLC) (Model 1100, Hewlett-Packard, Irvine, CA) using a C-18 reverse phase primesphere column 4.6 x 250 mm (Phenomenex, Torrance, CA). The mobile phase consisted of a gradient of 75% solution A to 25% solution B at time 0, which increased to 100% solution B at 12 min. Solution A was 0.1% trifluoroacetic acid, and solution B was 0.1% trifluoroacetic acid. The peaks were monitored at 280 and 550 nm.

The 1.9:1 lipid/polymer pharmaceutical composition, unencapsulated leuprolide solution and 0.9% NaCl solution were administered by a subcutaneous injection in the lateral lower back to 400 g male Harlan Sprague Dawley albino rats. The number of animals per group was six. The dose was 3.6 mg per rat and the volume was 600 FL. Serum was collected via venipuncture (saphenous vein)

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from unanesthetized animals at 0, 1.5 hour, 1, 3, 7, 14, 28, 60, 88, 92, 106 days. The time points collected for the unencapsulated leuprolide group were 0, 1.5 hour, 1, 3, 7, 14 days. The serum samples were kept at -40 °C until analysis.

Serum testosterone levels were determined with a solid-phase radioimmunoassay (RIA) kit. The Coat-A-Count total testosterone kit was purchased from Diagnostic Products Corporation (Los Angeles, CA). The assay is designed for the quantitative measurement of testosterone in serum. The assay is based on testosterone specific antibody immobilized to the wall of a polypropylene tube. ¹²⁵I-labeled testosterone competes for a fixed time with testosterone in the serum sample for antibody sites. The tube is then decanted, to separate the bound antibody from the free. The animal serum samples were counted in a gamma counter (Model 1175 Searle, Des Plaines, IL). The concentrations of serum testosterone were determined from a calibration curve, (log testosterone vs. log percent bound).

The serum testosterone concentrations (Fg/dL) at different time points after subcutaneous administration for the three treatment groups are shown in Fig. 11. Data from day 3 to day 106 are plotted on an expanded scale in Fig. 12.

Figs. 11 and 12 (an expanded vertical scale) show sustained release of leuprolide for up to 80-90 days after parenteral administration of leuprolide-containing lipid/polymer microspheres.

Example 12: Preparation of the Pharmaceutical Composition

A lipid/polymer-containing pharmaceutical composition with lipid:polymer ratio 1:1.12 was prepared as in Example 1 with the following modifications. The aqueous solution contained 80 mg/mL amikacin, pH 8. For preparing the lipid/polymer-containing pharmaceutical composition with lipid to polymer ratio of 1:1.12 by weight, the chloroform solution is as described in Example 2. The chloroform solution contained the polymer poly (lactide-co-caprolactone) (PLC) at a concentration of 50 mg/mL, DOPC, DPPG, cholesterol and triolein at concentrations of 10.4 mg/mL, 2.1 mg/mL, 7.7 mg/mL, 2.2 mg/mL,

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respectively. PLC was from Birmingham Polymers Inc. (Birmingham, AL), with a lactide: caprolactone ratio of 75:25. DOPC, DPPG, and triolein were from Avanti Polar Lipids (Alabaster, AL) and cholesterol was from Spectrum Chemical Manufacturing Corp. (Gardena, CA). The ratio of the volume of aqueous phase to the volume of volatile organic solvent phase was 5 mL to 5 mL. The water-in-oil emulsion was prepared by mixing on a Homo Mixer (Tokushu Kika Kogyo Co., Ltd., Osaka, Japan) at a speed of 9000 rpm for 10 min. The second aqueous phase was a mixture of 5 wt. % glucose and 40mM lysine. Spherules were formed by mixing at 6000 rpm for 1 min. A stream of nitrogen gas set at 50 psi for 20 minutes at 37 C was flushed through the suspension of spherules to evaporate the chloroform. The suspending medium was exchanged with 0.9 wt. % sodium chloride by washing and centrifuging at 600xg twice.

The particle diameter was measured using a laser scattering particle size distribution analyzer (Model LA-910, Horiba Instruments, Irvine, CA) using the length weighed distribution base and a relative refractive index of 1.18-1.00i. The mean particle diameter for the lipid/polymer pharmaceutical composition was 8.23 μm . The volume weighted mean particle size was 123.4 μm .

Example 13: Sustained Release *in vivo* of Encapsulated Physiologically Active Substance from Pharmaceutical Compositions after Interperitoneal Administration

The pharmaceutical compositions were prepared by employing a double emulsification process. The water-in-oil type emulsion was prepared from 5 mL of 25 mM arginine-glycine-glycine (Sigma, St. Louis, MO) buffer pH 7.9 containing 4 percent dextrose (Fisher, Fair Lawn, NJ), and erythropoietin with 2.5mg/mL human serum albumin (gift from UC San Diego Medical Center, San Diego, CA). The volatile organic phase contained lipids and polymer at a ratio of 1:1, with or without erythropoietin, or lipid/polymer ratio of 4:1 with erythropoietin. The water-immiscible solvent phase contained a mixture of phospholipids, cholesterol, triglycerides and PLGA (poly DL-lactide-co-glycolide). For the 1:1 (mass of lipid:mass of polymer) formulation, the solvent phase

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contained 10.4 mg/mL DOPC (dioleoyl phosphatidylcholine), 2.1 mg/mL DPPG (dipalmitoyl phosphatidylglycerol, sodium salt), 2.2 mg/mL triolein, all from Avanti Polar Lipids (Alabaster, AL), 7.7 mg/mL cholesterol (Spectrum, Garden, CA), and 22.4 mg/mL PLGA (Sigma, St. Louis, MO) dissolved in chloroform
5 (Spectrum, Gardena, CA). For the 4:1 formulation the weight ratio of lipid to polymer was varied, keeping the total concentration of lipid plus polymer constant. The mixing speed and time for producing the Awater-in-oil@ type emulsion was 9,000 rpm for 8 minutes on a Homo Mixer (Tokoshu Kika Kogyo Co., Ltd., Osaka, Japan). The second aqueous phase was 20 mL of a solution containing 4%
10 glucose (McGaw, Irvine, CA) and 40 mM lysine (Degussa, Courbevoie, France). The second aqueous phase was added to the Awater-in-oil@ suspension and mixed at 4,000 rpm for 1 minute. The organic solvent was removed by slow shaking at 37EC with nitrogen gas passing at 50 liters/minute for 20 minutes. The suspending medium was exchanged with 25mM arginine-glycine-glycine buffer pH 7.9 by
15 washing and centrifuging at 600xg twice.

The mean particle diameters were determined on a laser scattering particle size distribution analyzer (Model LA-910, Horiba Instruments, Irvine, CA). The mean particle diameters were 26.1 μm , 23.1 μm , 24.5 μm for the 1:1 and 4:1 lipid/polymer pharmaceutical compositions, and the 1:1 lipid/polymer blank
20 respectively.

The concentration of erythropoietin in the pharmaceutical composition was determined by quantitative colorimetric sandwich ELISA (R&D Systems, Minneapolis, MN) for human erythropoietin. Plates were read on an EL312E microplate, Bio-Kinetics reader (Bio-Tek Instruments, Winooski, VT) at 450 nm
25 wavelength for detection. The concentration of human serum albumin (HSA) in the blank was determined by high pressure liquid chromatography (HPLC) (Model 1100, Hewlett-Packard, Irvine, CA) using a C18 protein and peptide column 4.6 X 250 mm (Vydac, Hesperia, CA). The mobile phase consisted of a gradient of 5% solution A to 80% solution B in 18 minutes. Solution A was 0.1% trifluoroacetic
30 acid (TFA)(Pierce, Rockford, IL) in HPLC water from Mallinckrodt (Paris, KY).

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and solution B was 0.1% TFA in methanol from Burdick & Jackson (Muskegon, MI) with a flow rate of 1 mL per minute. The peaks were monitored at 280 nm wavelength.

Concentration of the pharmaceutical composition and unencapsulated erythropoietin were adjusted to 500 IU of erythropoietin with arginine-glycine-glycine buffer at pH 7.9. Blank was adjusted to contain 0.4 mg/mL of HSA protein. Five female CD1/ICR Harlan Sprague Dawley mice weighing 28-32 grams were injected intraperitoneal (IP) for each pharmaceutical composition. The dose was 500 IU erythropoietin in a 1 mL total volume. Blood was collected via the orbital sinus from animals anesthetized with 2.5% Halothane and 1L/minute oxygen. Hematocrits were taken on days 0, 1, 3, 6, 10, 13, 17, 21, 24. Blood was collected directly into capillary tube and spun for 6 minutes on a hematocrit centrifuge.

The results in Figure 13 show sustained effect of hematocrit for up to 13 days with the 4:1 lipid/polymer pharmaceutical composition and up to 10 days for the 1:1 lipid/polymer pharmaceutical composition.

The increase in hematocrit resulting from an injection of free (unencapsulated) erythropoietin is not as pronounced as with the encapsulated drug. Injected free (unencapsulated) erythropoietin resulted in this effect lasting only up to 6 days. The 4:1 lipid:polymer composition shows a 15.6 percent increase in hematocrit in comparison to unencapsulated drug. The 1:1 pharmaceutical composition shows a 7.8 percent in hematocrit in comparison to unencapsulated drug. The 4:1 lipid:polymer pharmaceutical composition resulted in the highest hematocrit overall.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A lipid/polymer-containing pharmaceutical composition comprising:
a biodegradable microsphere having a matrix, said matrix comprising at
least one type of biodegradable polymer, and at least one type of lipid; and
5 a physiologically active substance which is releasable from the
biodegradable microsphere.
2. The pharmaceutical composition of claim 1, wherein the microsphere is
substantially free of volatile organic solvent.
3. The pharmaceutical composition of claim 1, wherein the microsphere is
10 substantially free of poly vinyl alcohol.
4. The pharmaceutical composition of claim 1 in the form of a solid dosage.
5. The pharmaceutical composition of claim 4, wherein the solid dosage is selected
from the group consisting of tablets, capsules, wafers, transdermal patches,
sutures, implants, and suppositories.
- 15 6. The pharmaceutical composition of claim 1 in the form of an aqueous
suspension.
7. The pharmaceutical composition of claim 1, wherein at least one of the
biodegradable polymer types is a homopolymer.

8. The pharmaceutical composition according to claim 7, wherein at least one of the biodegradable homopolymers is selected from the group consisting of polylactides, polyglycolides, poly(p-dioxanones), polycaprolactones, polyhydroxyalkanoates, polypropylenefumarates, polyorthoesters, polyphosphate
5 esters, polyanhydrides, polyphosphazenes, polyalkylcyanoacrylates, polypeptides, and genetically engineered polymers.
9. The pharmaceutical composition of claim 1, wherein at least one of the biodegradable polymer types is a random or block copolymer.
10. The pharmaceutical composition of claim 9, wherein at least one of the
10 biodegradable random or block copolymers is selected from the group consisting of poly(lactide-glycolides), poly(p-dioxanone-lactides), poly(p-dioxanone-glycolides), poly(p-dioxanone-lactide-glycolides), poly(p-dioxanone-caprolactones), poly(p-dioxanone-alkylene carbonates), poly(p-dioxanone-alkylene oxides), poly(p-dioxanone-carbonate-glycolides), poly(p-dioxanone-carbonates), poly(caprolactone-lactides), poly(caprolactone-glycolides),
15 poly(hydroxyalkanoates), poly(propylenefumarates), poly(orthoesters), poly(ether-esters), poly(ester-amides), poly(ester-urethanes), polyphosphate esters, polyanhydrides, poly(ester-anhydrides), polyphosphazenes, polypeptides and genetically engineered copolymers.
- 20 11. The pharmaceutical composition of claim 1, wherein at least one of the lipid types comprises a zwitterionic lipid.
12. The pharmaceutical composition of claim 1, wherein at least one of the lipid types comprises an acidic lipid.
13. The pharmaceutical composition of claim 1, wherein at least one of the
25 lipid types comprises a sterol.

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14. The pharmaceutical composition of claim 1, wherein at least one of the lipid types comprises a triglyceride.
15. The pharmaceutical composition of claim 1, wherein the physiologically active substance is hydrophilic, and wherein the composition further comprises
5 a triglyceride.
16. The pharmaceutical composition of claim 1, wherein the physiologically active substance is hydrophobic, and wherein the composition is free of triglycerides.
17. The pharmaceutical composition of claim 1, wherein the physiologically
10 active substance is selected from the group consisting of antianginas, antiarrhythmics, antiasthmatic agents, antibiotics, antidiabetics, antifungals, antihistamines, antihypertensives, antiparasitics, antineoplastics, antivirals, cardiac glycosides, herbicides, hormones, immunomodulators, monoclonal antibodies, neurotransmitters, nucleic acids, proteins, radio contrast agents,
15 radionuclides, sedatives, analgesics, steroids, tranquilizers, vaccines, vasopressors, anesthetics, peptides, and combinations thereof.
18. A method of controlling the rate of release of a physiologically active substance upon administration to a patient by varying the lipid to polymer ratio in the pharmaceutical composition of claim 1.

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19. A process for producing biodegradable lipid/polymer-containing compositions, the process comprising:
- forming a Awater-in-oil@ type emulsion from a first aqueous phase and a volatile organic solvent phase comprising at least one type of volatile organic solvent, at least one type of biodegradable polymer or copolymer, and at least one type of lipid;
 - dispersing the Awater-in-oil@ type emulsion into a second aqueous phase to form solvent spherules; and
 - removing the volatile organic solvent from the solvent spherules to form a lipid/polymer-containing composition suspended in the second aqueous phase.
20. A process for producing biodegradable lipid/polymer-containing pharmaceutical compositions, the process comprising:
- (a) forming a Awater-in-oil@ type emulsion from a first aqueous phase comprising at least one hydrophilic physiologically active substance, and a volatile organic solvent phase comprising at least one volatile organic solvent, at least one type of biodegradable polymer or copolymer, and at least one type of lipid;
 - (b) dispersing the Awater-in-oil@ type emulsion into a second aqueous phase to form solvent spherules; and
 - (c) removing the volatile organic solvent from the solvent spherules to form a lipid/polymer-containing pharmaceutical composition suspended in the second aqueous phase,
- wherein the lipid:polymer ratio is chosen to modulate the *in vivo* release rate of the physiologically active substance.
21. The process of claim 20, further comprising removing substantially all of the organic solvent from the solvent spherules.
22. A pharmaceutical composition produced by the process of claim 20.

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23. A process for producing biodegradable lipid/polymer-containing pharmaceutical compositions, the process comprising:
- (a) forming a water-in-oil type emulsion from a first aqueous phase, and a volatile organic solvent phase comprising at least one hydrophobic physiologically active substance, at least one volatile organic solvent, at least one type of biodegradable polymer or copolymer, at least one type of lipid;
 - (b) dispersing the water-in-oil type emulsion into a second aqueous phase to form solvent spherules; and
 - (c) removing the volatile organic solvent from the solvent spherules to form a lipid/polymer-containing pharmaceutical composition suspended in the second aqueous phase,
- wherein the lipid:polymer ratio is chosen to modulate the *in vivo* release rate of the physiologically active substance.
24. The process of claim 23, further comprising removing substantially all of the organic solvent from the solvent spherules.
25. A lipid/polymer-containing pharmaceutical composition produced according to the process of claim 23.
26. A method of treating a disorder in living beings, the method comprising administering a therapeutically effective amount of the lipid/polymer-containing pharmaceutical composition of claim 1 to a subject afflicted with the disorder, wherein the pharmaceutical composition comprises a physiologically active substance which is effective in treatment of the disorder.
27. The method of claim 26, wherein the administration is a parenteral administration.
28. The method of claim 26, wherein the administration is an enteral

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administration.

29. The method of claim 27, wherein the physiologically active substance is a hormone
30. The method of claim 29, wherein the hormone is insulin or leuprolide.
- 5 31. The method of claim 27, wherein the physiologically active substance is a cytokine.
32. The method of claim 31, wherein the cytokine is erythropoietin.
33. The method of claim 27, wherein the physiologically active substance is an antibiotic.
- 10 34. The method of claim 33, wherein the antibiotic is amikacin.
35. The process of claim 20, wherein the volatile organic solvent phase further comprises at least one tryglyceride.

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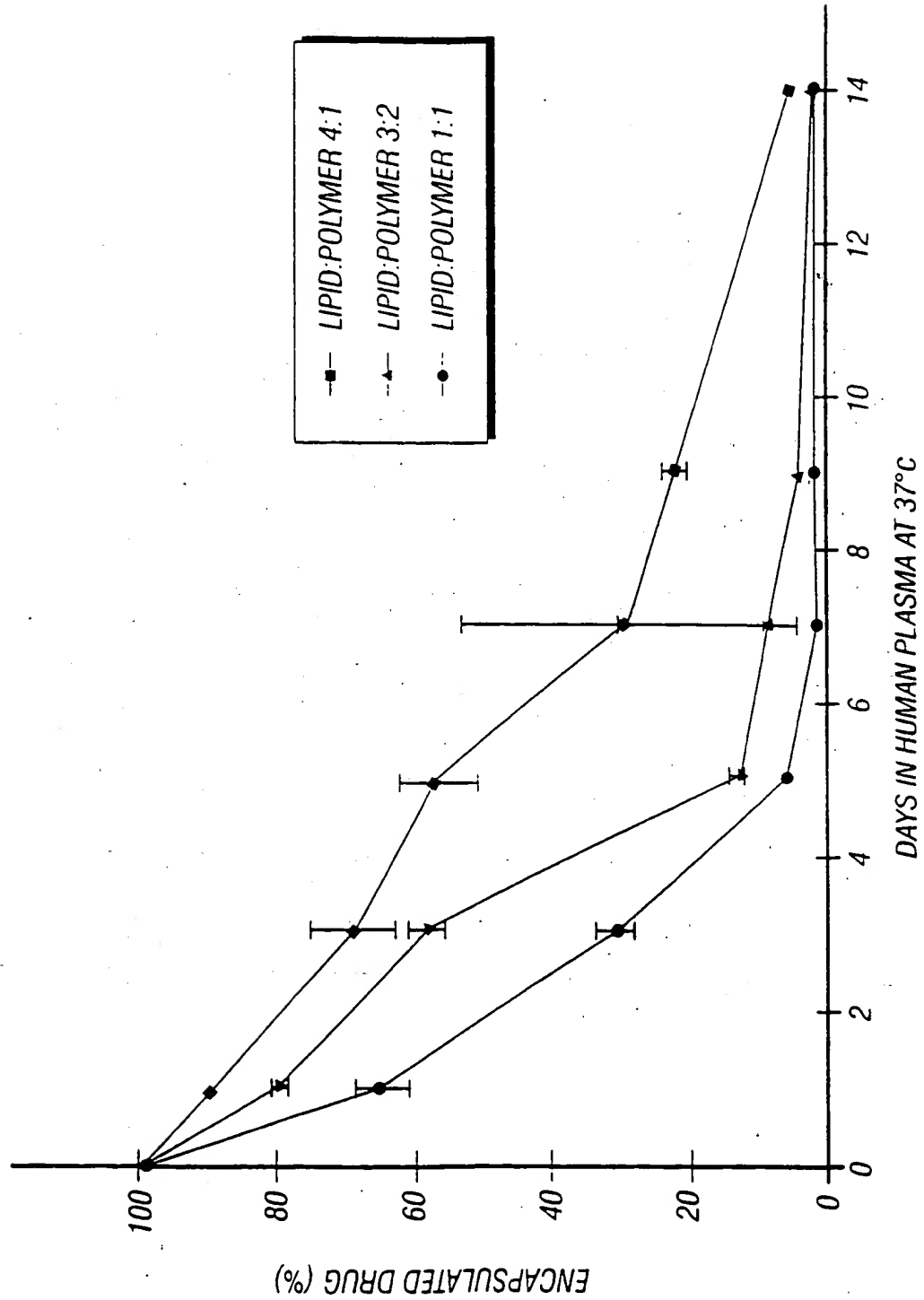


FIG. 1

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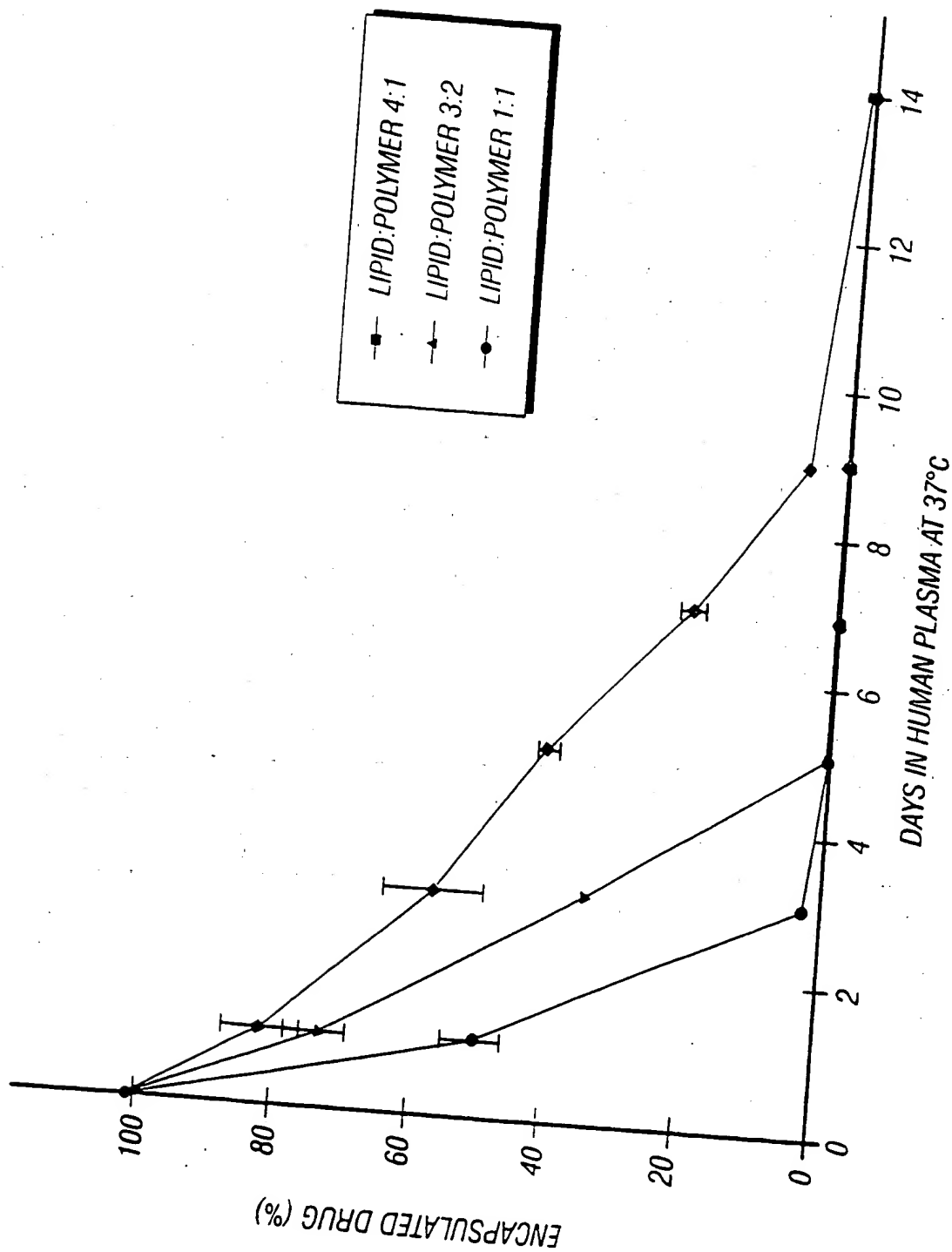


FIG. 2

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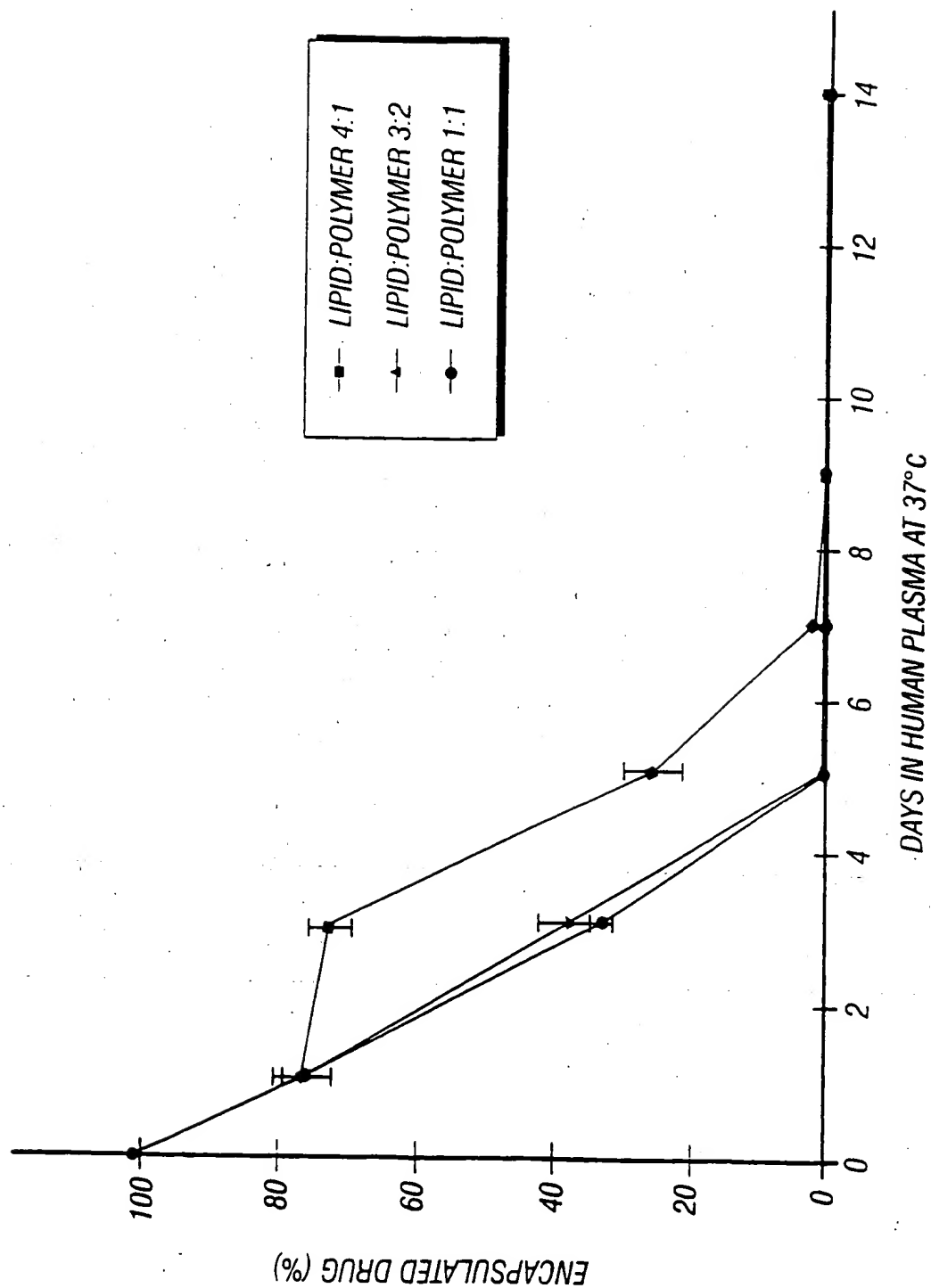


FIG. 3

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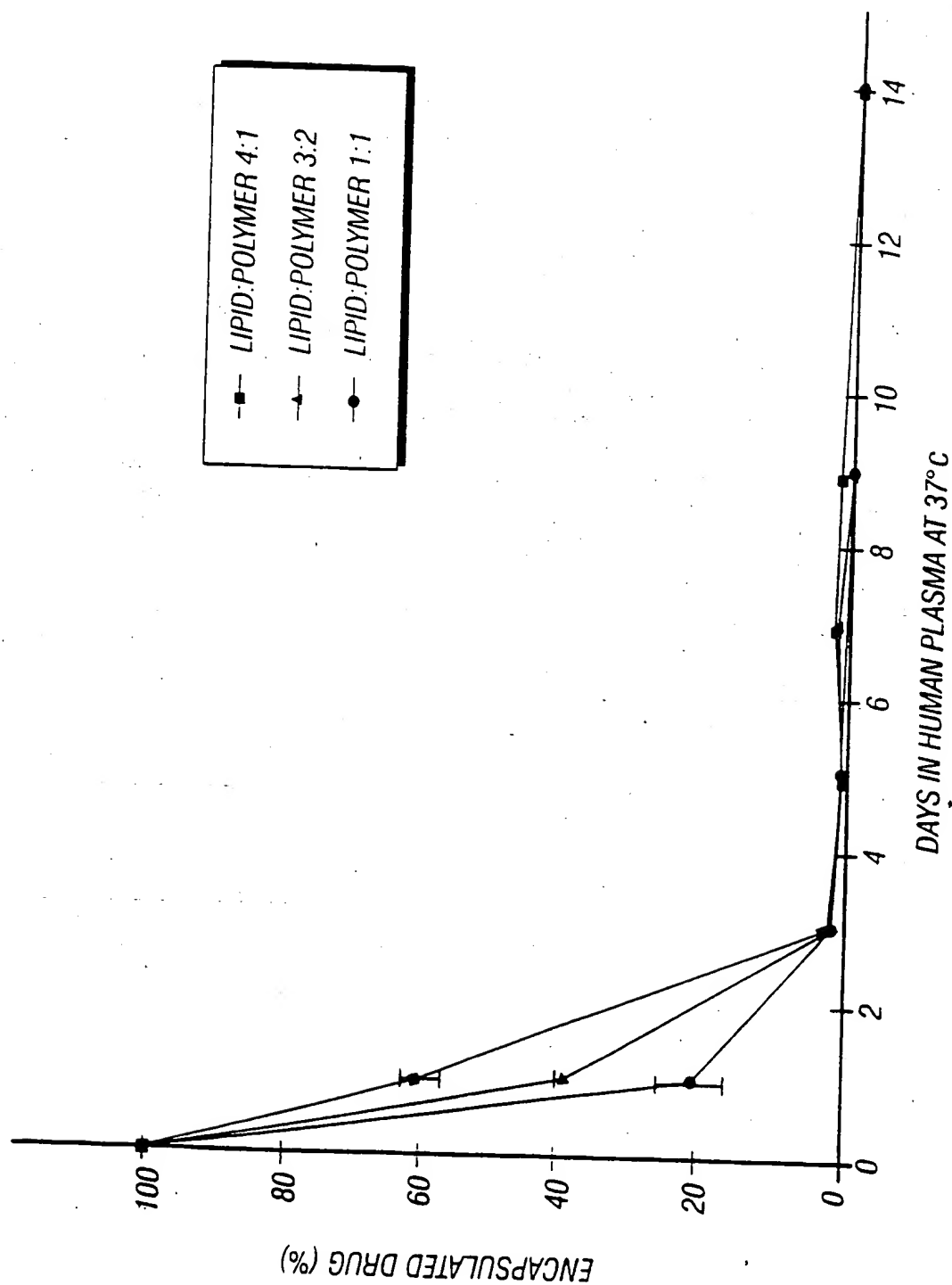


FIG. 4

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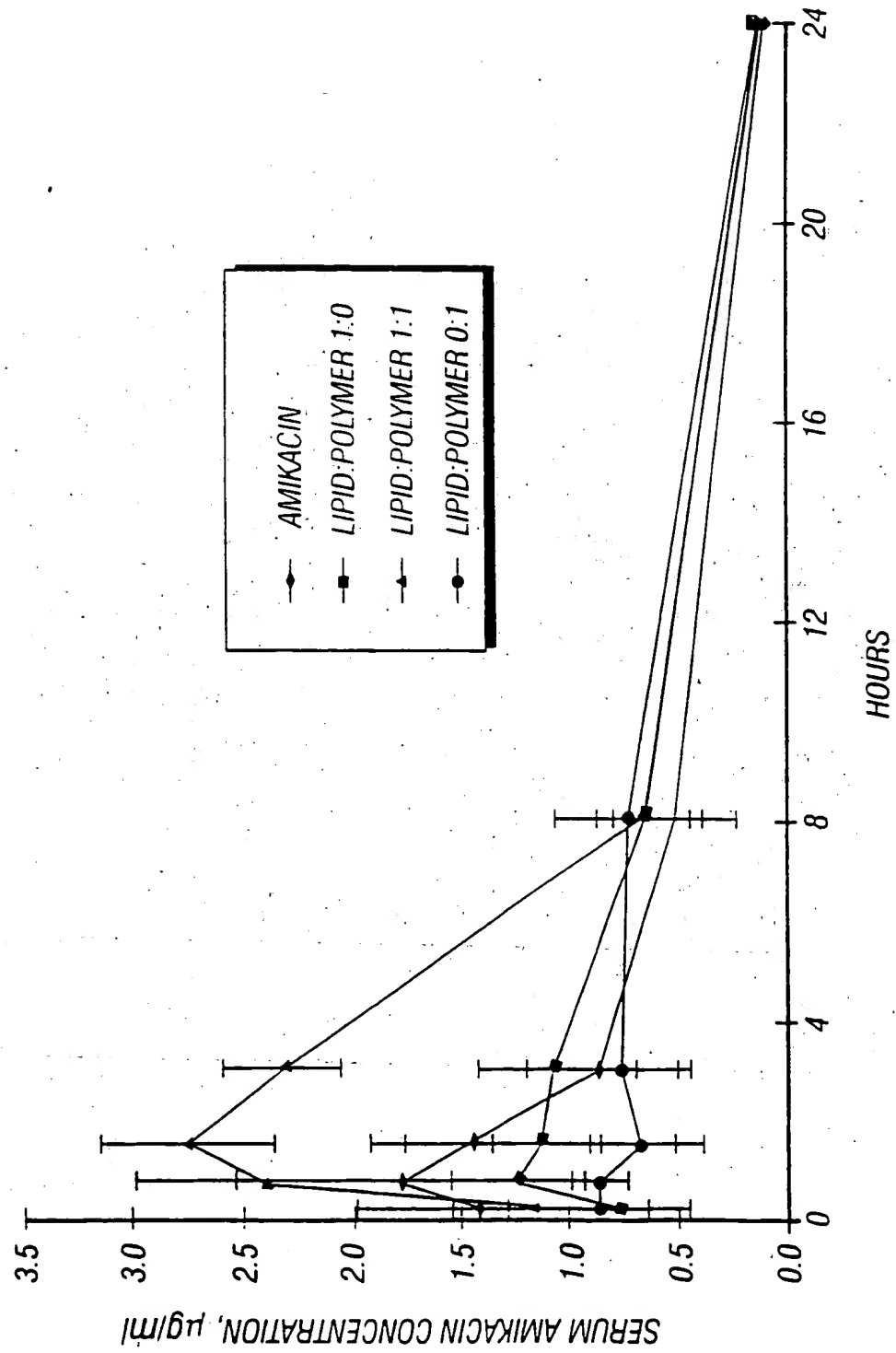


FIG. 5

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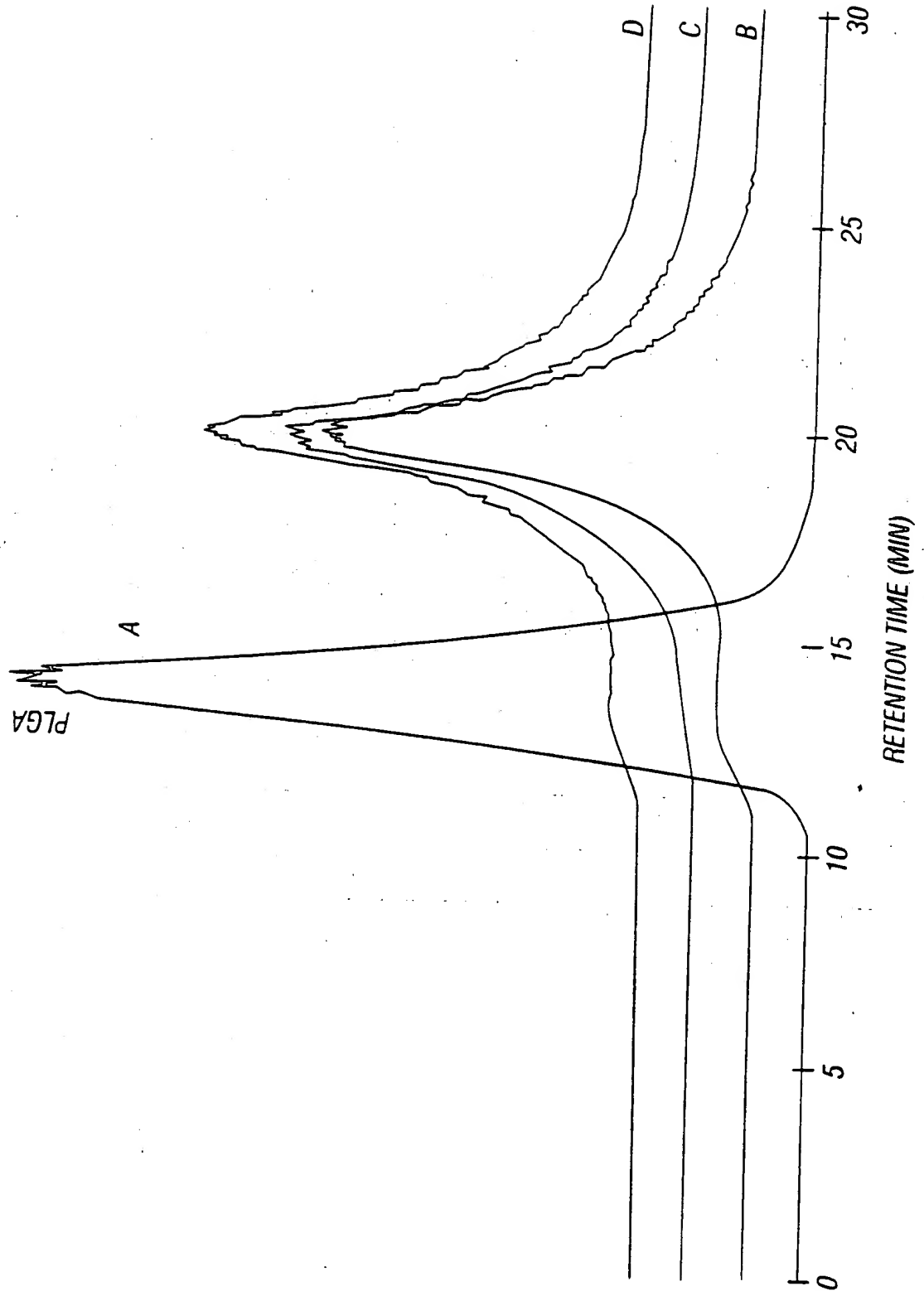


FIG. 6

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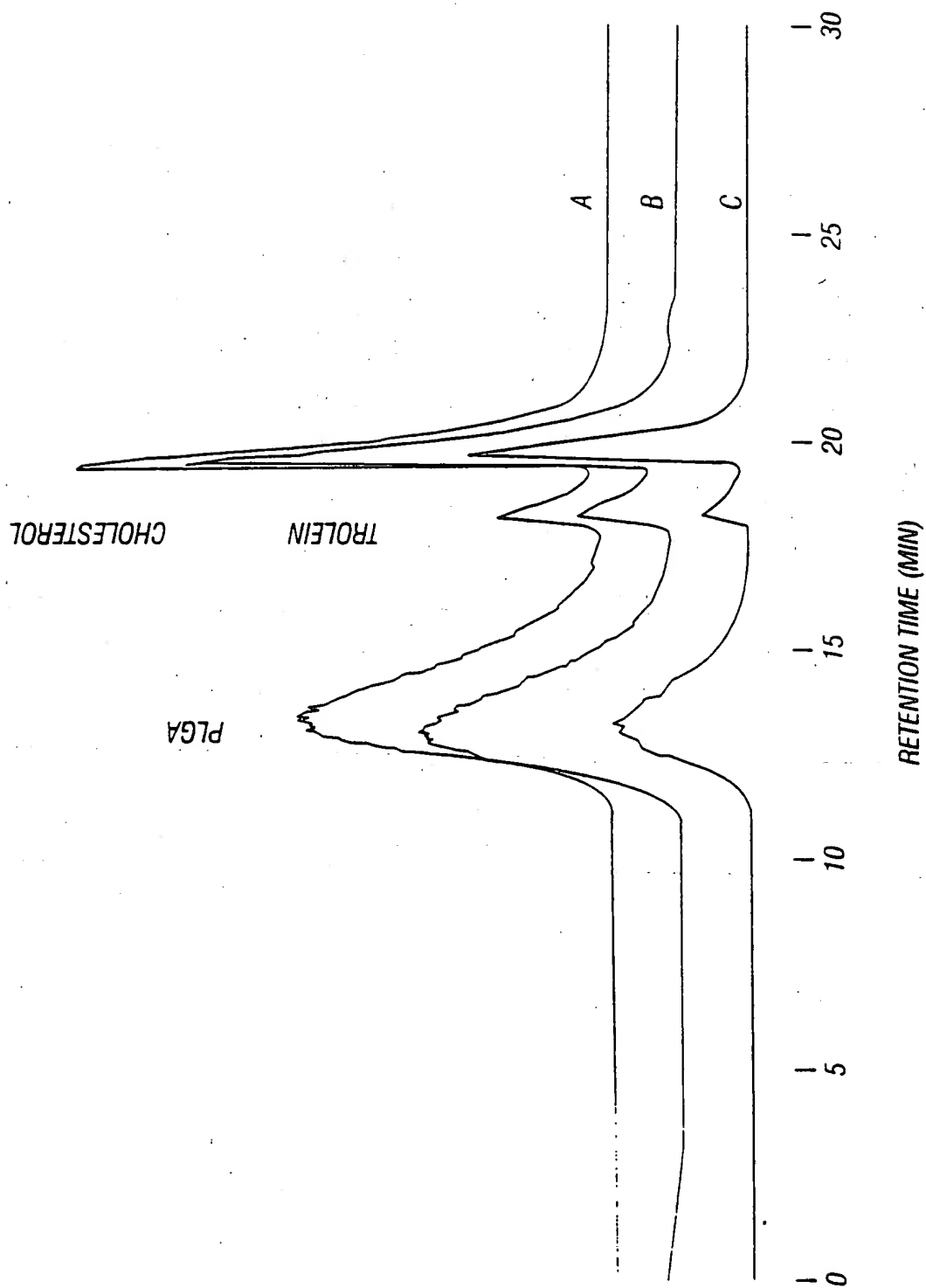


FIG. 7

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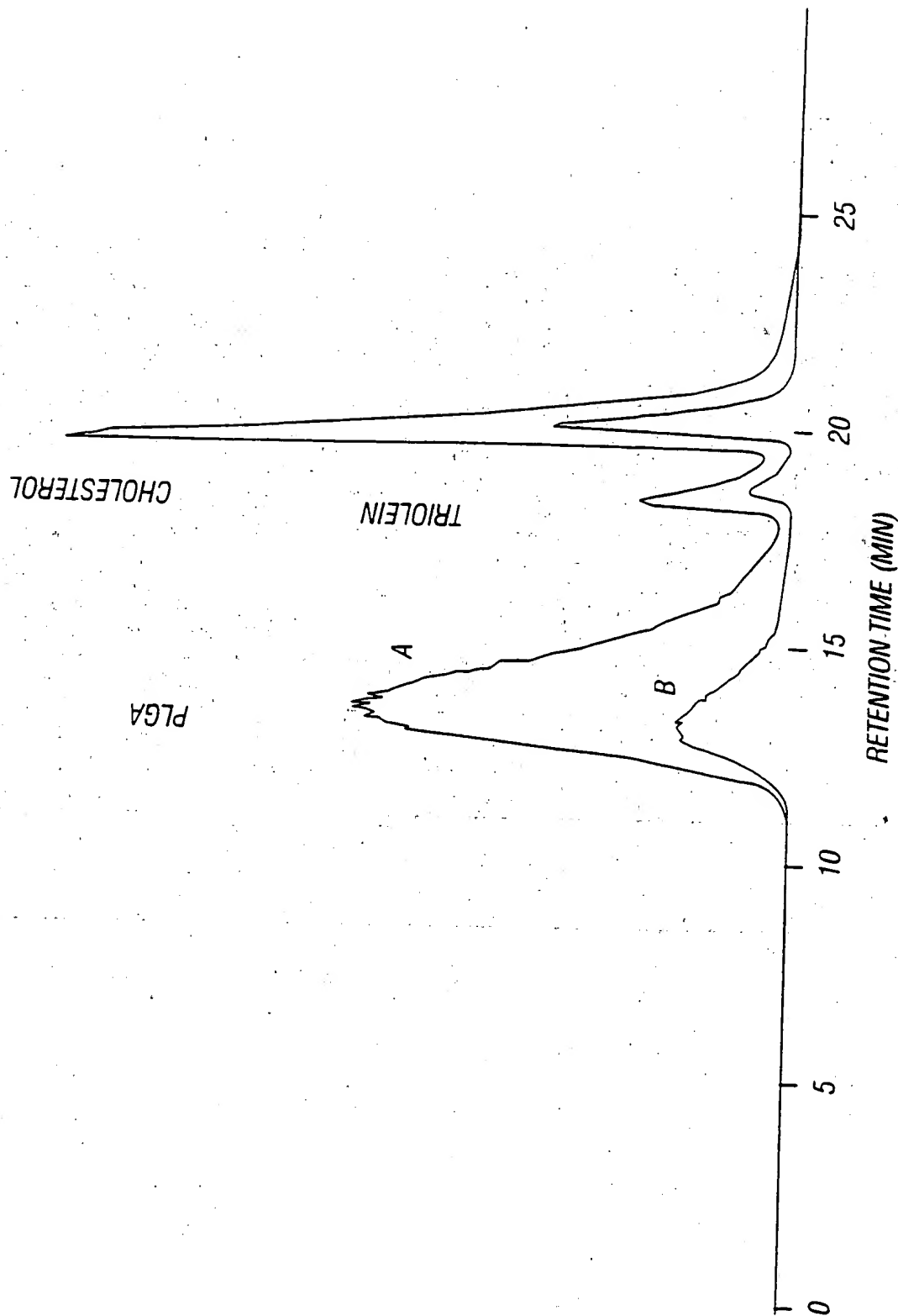
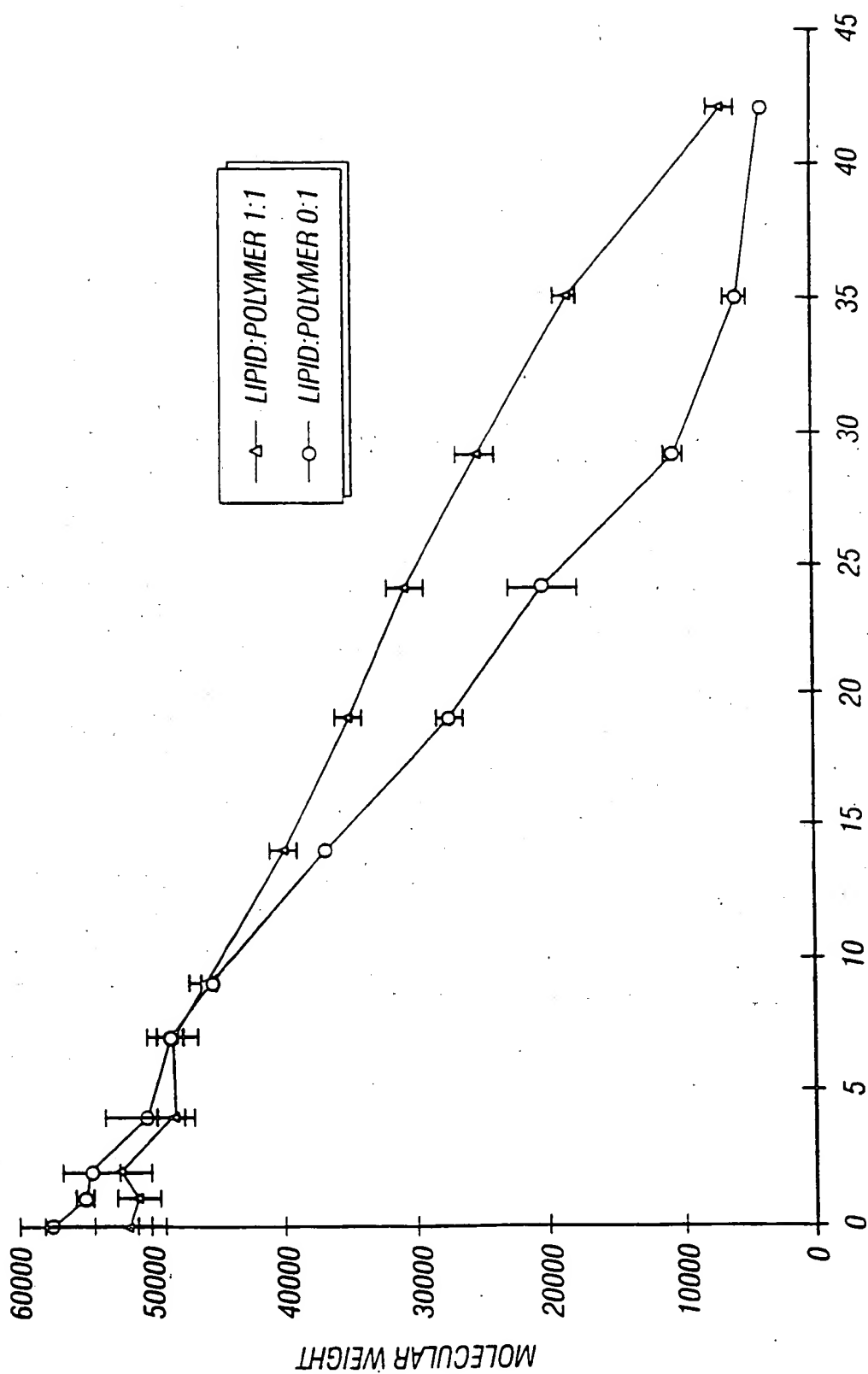


FIG. 8

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DAYS OF INCUBATION AT 37°C

FIG. 9

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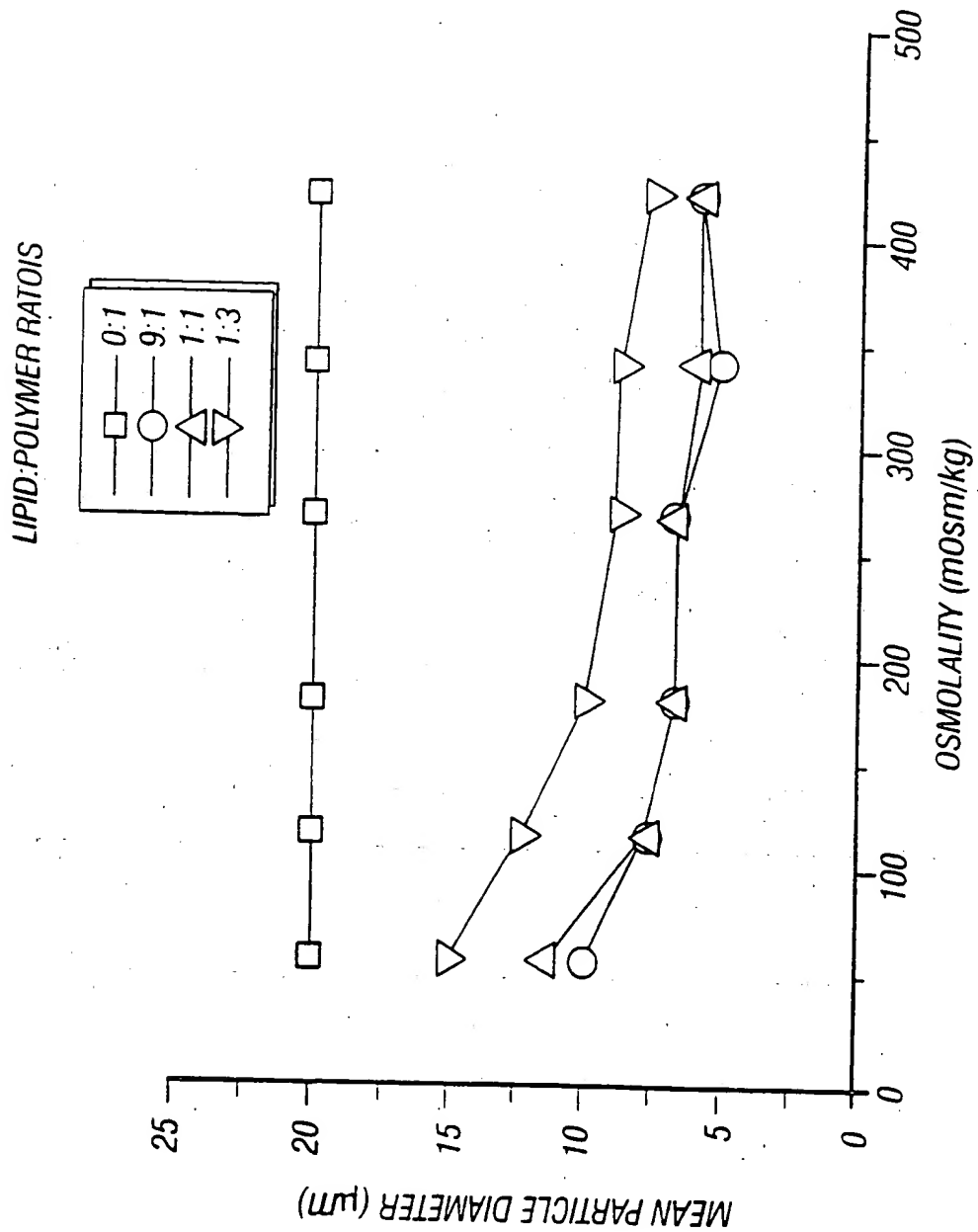


FIG. 10

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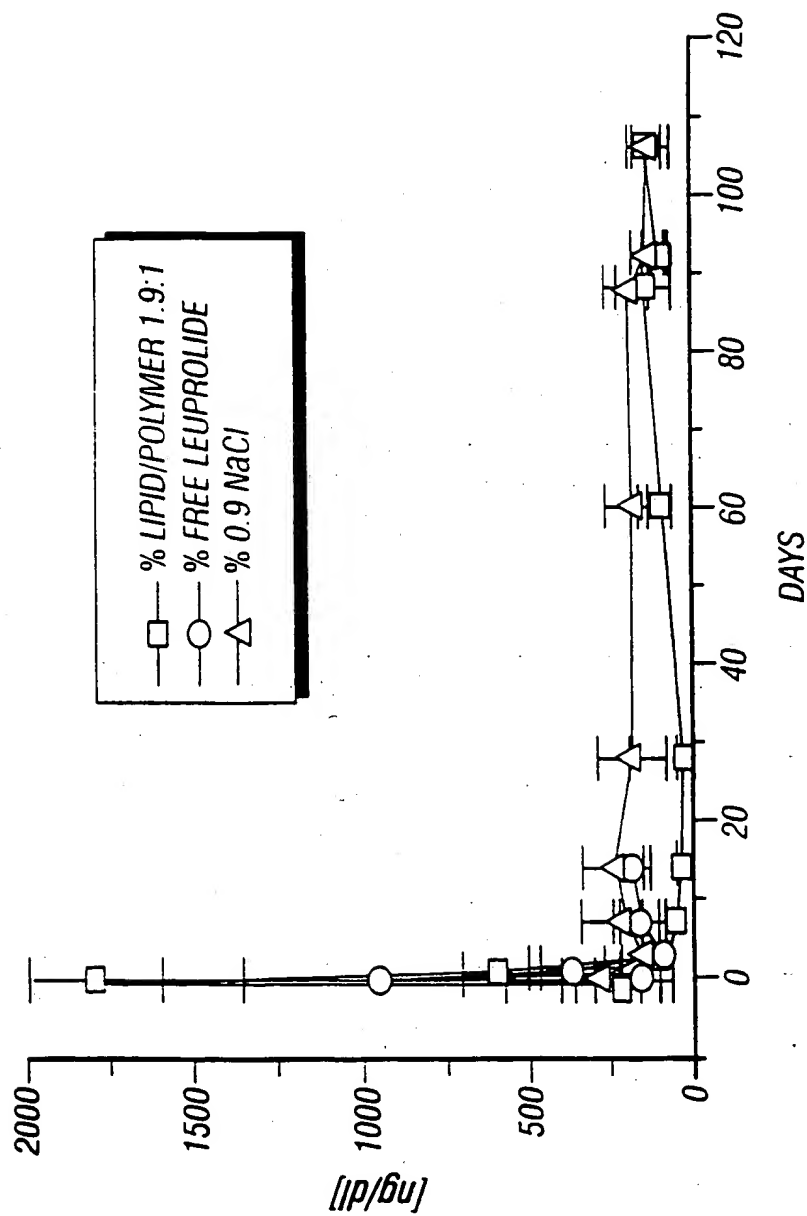


FIG. 11

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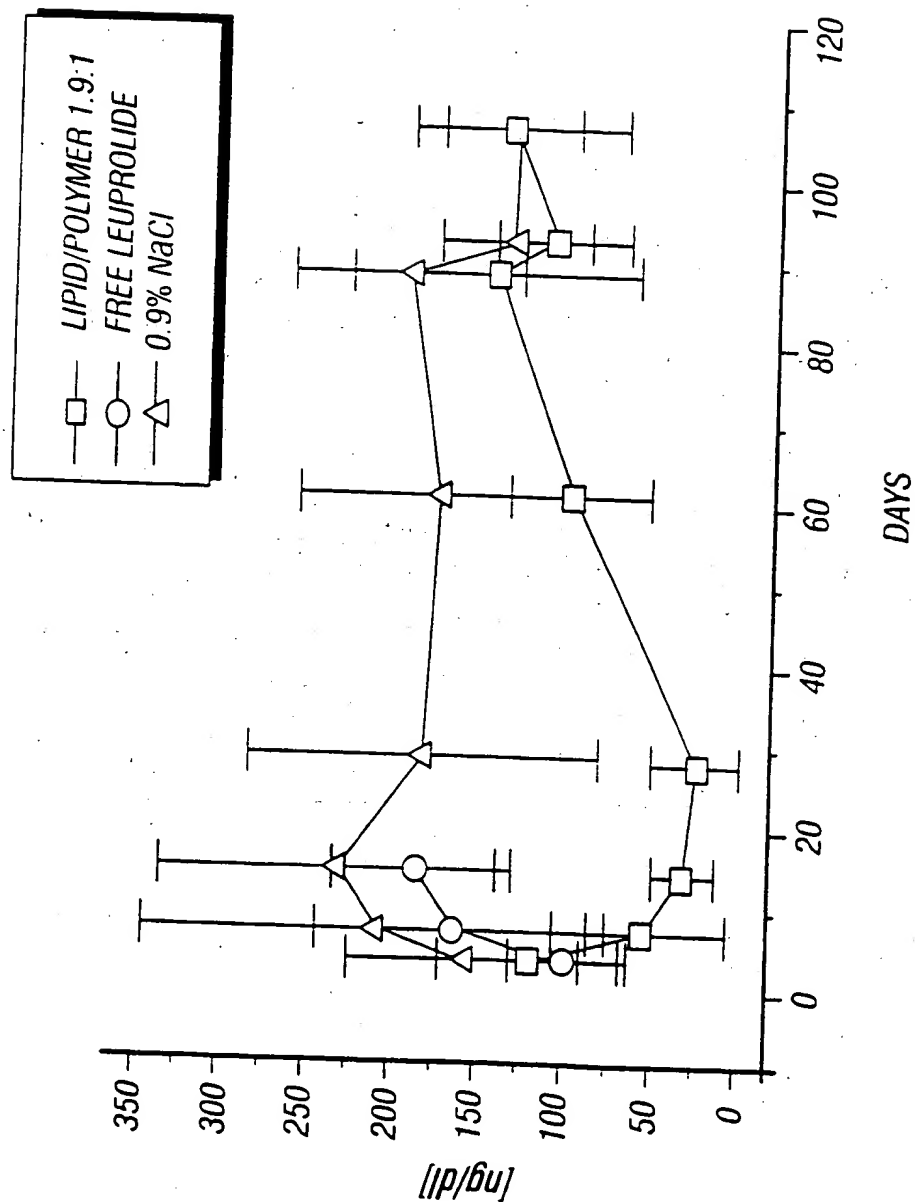


FIG. 12

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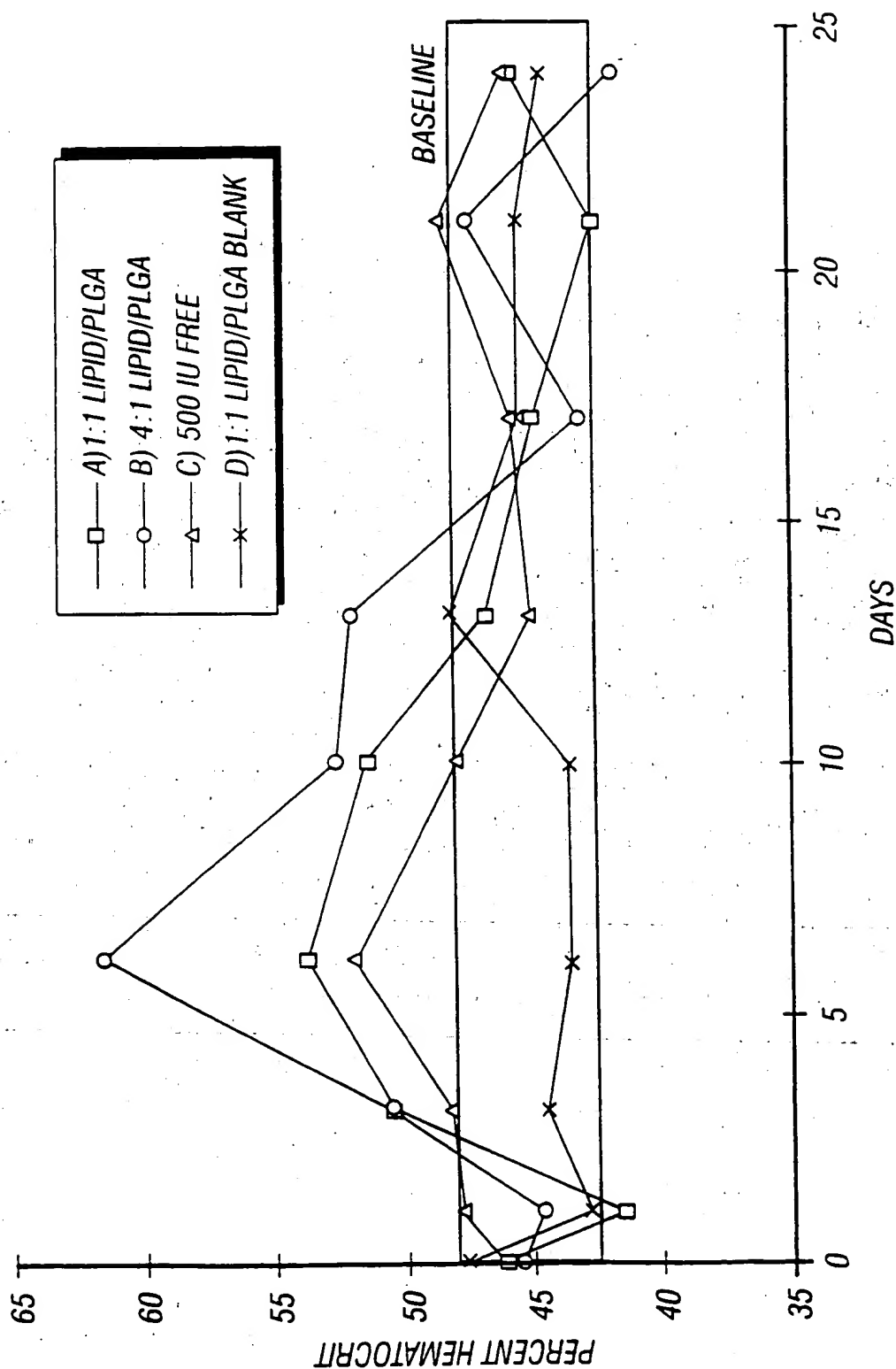


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16239

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61F 2/02, 13/02; A61K 9/20, 9/48, 9/50
US CL : 424/423, 433, 439, 449, 451, 464, 501, 502

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/423, 433, 439, 449, 451, 464, 501, 502

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,543,158 A (GREF ET AL) 06 AUGUST 1996, see entire document.	1-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 SEPTEMBER 1999

Date of mailing of the international search report

20 OCT 1999

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